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Trophic Dynamic Interactions in a Temperate Karst River

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TROPHIC DYNAMIC INTERACTIONS IN A TEMPERATE KARST RIVER

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Surface streams in karst landscapes are often characterized by high nutrient levels due to incomplete filtration through series of innumerable, below-ground conduits. Seasonal growth of the filamentous alga, *Cladophora*, is typically associated with nutrient-rich waters. This research compared macroinvertebrate food web structure between riverine reaches with contrasting underlying karst topography, nutrient levels, and *Cladophora* cover during summer 2012 and autumn 2013. Recent work in these reaches found a high correlation between *Cladophora* cover and nutrient content, particularly nitrate. Four questions were addressed during this study:

1. Do longitudinal trends in algal and consumer δ13C values relate to decreased DIC availability in larger watersheds?
2. Are trophic niche breadths narrower in more karstified reaches than in less karstified reaches due to longitudinal differences in *Cladophora* standing stocks?
3. Do differences in trophic-dynamic relationships between primary consumers and their food resources reflect the marked distinction in *Cladophora* standing stocks in two sections of the upper Green River that flow through differing levels of karstification?
4. Are consumers assimilating primarily autochthonous or allochthonous food resources?

Consumers and algae became more ^{13}C-depleted in downstream reaches, which is opposite to published data in other streams. Underlying causes for this pattern are uncertain, but one plausible cause is an increase in DIC availability downstream. Karst-related hydrology may potentially alter or even reverse normal longitudinal gradients within in-stream producer and subsequently, consumer $\delta^{13}C$ values.

Since consumers were sampled during low-*Cladophora* conditions during 2013 and within a few weeks of the onset of the *Cladophora* bloom in 2012, stable isotopic results may be more representative of primary consumer diets during pre-*Cladophora* bloom periods. Although *Cladophora* cover was significantly higher in downstream reaches during both years, food-web structure was similar in all reaches. Consumer niche breadth was similar across reaches, and mixing model analyses suggested that primary consumers in all reaches assimilated similar amounts of *Cladophora*. The contribution of both autochthonous and allochthonous food resources to the assimilated diet of primary consumers appeared to be similarly important. These results suggest that allochthonous resources may be important in some midreach food webs, especially during periods of low algal growth.

CHAPTER 1: STUDY BACKGROUND

Introduction

Trophic-dynamics, or the transfer of carbon and energy from one organism to another, remains a well-studied central theme of ecology (Pimm 1982; DeAngelis 1992; Polis et al. 1997; Doi 2009). Food web studies provide insight into the effects that community structure and underlying ecosystem processes have on member organisms (Polis et al. 1997; Doi 2009). In aquatic systems, many studies attempt to distinguish energy derived from in-stream primary producers (autochthonous resources) from terrestrial-derived subsidies (allochthonous resources) (Vannote et al. 1980; Doucett et al. 2007).

Food web structures in aquatic systems are often spatially heterogeneous because of gradients in primary productivity patterns, resource abundance, and community composition of primary consumers (Polis et al. 1997; Shindler & Scheuerell 2002; Doi 2009). In particular, upstream to downstream gradients in the availability of food resources may be affected by differences such as channel size, hydrology, and algal productivity in streams, which can influence consumer diets (Vannote et al 1980; Power & Dietrich 2002). While often overlooked in stream ecology studies, underlying lithology has the potential to influence both flow patterns and the chemical composition of stream water (Edmond & Huh 1997; Winter 1999; Schulte et al. 2011). Longitudinal gradients in underlying lithology, therefore, may be indirectly related to longitudinal gradients in chemical composition of stream water and algal accrual (Notestein et al. 2003; Penick et al. 2012).

Karst, carbon, and primary productivity

Lithology greatly influences the biogeochemistry of riverine systems (Edmond & Huh 1997; Winter 1999; Schulte et al. 2011). Dissolved and particulate minerals from weathered bedrock can change the mineral content and pH of surface streams (Newton et al. 1987; Berner & Berner 1997). This weathering process can occur through physical abrasion, biological means, or chemical dissolution (Ollier 1984; Bennett et al. 2001).

Karst geology refers to a landscape dominated by bedrock that is soluble by weak acid, typically derived from water interacting with carbon dioxide (CO_2) (Yuan 1988). The development of karst landscapes is largely caused by dissolution rather than physical abrasion (Ford & Williams 2007). This type of landscape is common globally and throughout the United States. Karst landscapes cover roughly 20% of the Earth's ice-free land area, and groundwater from karst areas provide drinking water for up to 25% of the world's human population (Ford et al. 1988; Williams & Fong 2010). Approximately 40% of the U.S is karst landscape (White et al. 1995; Ford & Williams 2007).

The vast majority of karst landscapes are comprised mainly of carbonate rocks, such as limestone or dolostone, though a few karst landscapes are composed of other types of rock (Palmer 2007). Carbonate rocks weather much faster than silicate rocks (Roy et al. 1999). Because of their relatively fast rate of weathering, carbonate rocks have a greater influence than silicate rocks on riverine calcium and dissolved inorganic carbon (DIC) concentrations even when they compose only a relatively small fraction of the lithology in a watershed (Roy et al. 1999).

Limestone is composed of crystalline calcium carbonate. Meteoric water combines with atmospheric CO_2 to form H_2CO_3 (Drever 1982). Processes within the soil,

including plant root respiration and microbial activity, further provides CO_2 that is available to acidify water as it percolates down through the soil column. The resulting acidic water (carbonic acid) dissolves calcite through the reaction:

$$H_2O + CO_2 + CaCO_3 \leftrightarrow Ca^{2+} + 2HCO_3^-$$

(Drever 1982; White & White 1989; Liu & Zhao 2000).

Limestone bedrock may directly alter riverine systems with dissolved minerals resulting from the weathering process, most notably through inputs of inorganic carbon (Schulte et al. 2011). DIC is the most abundant form of carbon in riverine systems (Brunet et al. 2009). Although several other factors also influence DIC concentrations, such as photosynthesis, respiration, and interaction with the atmosphere, groundwater inputs are a prominent source of this mineral (Redfield 1958; Kling et al. 1992; Cox et al. 2000; Friedlingstein et al. 2001; Hope et al. 2001; Doctor et al. 2008; Schulte et al. 2011).

While groundwater inputs in karst landscapes often are DIC-rich, high pH associated with alkaline waters in karst landscapes changes the relative abundance of the different species of inorganic carbon in the river. Natural waters typically have a pH between 6.5 and 8.5, although diurnal variations may exist due to biological processes (e.g., respiration; Hem 1985). Inorganic carbon in water exists in three interconvertible species: carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (H_2CO_3). CO_2 is the dominant form of inorganic carbon in water in acidic water (Hutchinson 1957). CO_2 and HCO_3^- concentrations are equal at a pH of 6.4. At a neutral pH, only around 10% of DIC is in the form of CO_2 and the vast majority of remaining DIC is in the form of bicarbonate. Carbonate is present in waters with a pH higher than 6.5, but rarely makes up more than 1% of DIC even in most naturally occurring alkaline streams. Surface

streams with a relatively high pH because of karst groundwater inputs will have most of their DIC in the form of bicarbonate. Most of the DIC available to in-stream primary producers in karst areas, therefore, will be in the form of HCO_3^- rather than CO_2.

Aquatic plants and algae fix DIC into organic forms via photosynthetic pathways. Access to DIC by aquatic producers is determined by two factors: DIC abundance and, in environments with limited DIC abundance, water velocity. Carbon limitation can occur even in relatively DIC-rich environments if autochthonous producers are surrounded by a carbon depleted layer of stagnant water or encased within a periphyton matrix, which prevents CO_2 from diffusing into the plant or algae (Keeley & Sandquist 1992; Hill & Middleton 2006). In contrast, the effects of low carbon dioxide concentrations may be mediated by high water velocity in some streams. Lotic environments with higher water velocity decrease the thickness of the stagnant layer of water surrounding primary producers, thereby increasing the exposure of the primary producer to CO_2 (Keeley & Sandquist 1992; Finlay et al. 1999).

Some primary producers may also be carbon limited when the pH of stream water is alkaline, since most of the inorganic carbon is in the form of HCO_3^- (Raven et al. 1985; Finlay 2003). Primary producers need a high CO_2 concentration inside the cell to effectively photosynthesize. During photosynthesis, RuBisCO binds to CO_2 but is unable to bind to HCO_3^-. However, RuBisCO has a low specificity for CO_2 and often binds to oxygen instead when CO_2 concentrations are too low. This process is called photorespiration and is energy inefficient for the cell (Keys 1986). Some types of algae and plants have no additional mechanisms other than passive diffusion to concentrate CO_2 inside the cell. Some studies have suggested that these taxa, such as bryophytes and

some types of red algae may be especially impacted in areas where most available DIC is in the form of HCO_3^- (Raven et al. 1985; Finlay 2003).

Many stream primary producers circumvent this potential carbon limitation using carbon concentrating mechanisms. Carbonic anhydrase (CA) is a metalloenzyme that catalyzes the reversible reaction between HCO_3- and CO_2 (Khalifah 1971; Smith & Ferry 2000). CA greatly increases the rate at which one inorganic carbon species is converted to another in the following reactions:

$$CO_2 + H_2O \rightarrow H_2CO_3^-$$

or

$$HCO_3^{--} + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O^-$$

(Khalifah 1971; Lindskog 1997). Primary producers use carbon concentrating mechanisms (CCMs) to increase levels of CO_2 around RuBisCO through a number of different specialized mechanisms, which minimizes photorespiration (Giordano et al. 2005). Many of these mechanisms use CA either inside or outside the cell membrane. Some of the most common forms of CCMs involve external CA, active transport of HCO_3^- into the cell, and proton pumps. Some algae actively transport HCO_3^- into the cell (Palmqvist et al. 1994; Amoroso et al. 1998). The HCO_3^- in the cell passively diffuses to the thylakoid lumen, which has been acidified by proton pumps. This causes some of the HCO_3^- to convert to CO_2. Then the CO_2 passes through a membrane to the compartment where RuBisCO is located (Giordano et al.2005). Other types of algae secrete CA outside of the cell, which then converts HCO_3^- to CO_2 near the cell membrane. CO_2 then passively diffuses into the cell (Fujiwara et al. 1990; Rawat & Moroney 1991).

These mechanisms are inefficient in terms of energy cost, however, and most aquatic plants and algae preferentially take up CO_2 over HCO_3^- (Giordano et al. 2005). Energy levels in the cell, nutrient supplies, and CO_2 levels can all influence the expression of CCMs in algae (Giordano et al. 2005). Most primary producers with CCMs upregulate CCM activity under low CO_2 conditions when the benefit of converting HCO_3^- is greater than the energy cost of using CCMs (Giordano et al. 2005).

Other studies have speculated that highly alkaline systems found in karst areas may influence the primary producer communities by creating environments where most DIC is in the form of HCO_3^-, which would favor species that have carbon concentrating mechanisms (Raven et al. 1985; Finlay 2003). While carbon limitation is unlikely for most species in karst areas due to high inputs of DIC in groundwater, most aquatic species may greatly benefit from CCMs, which convert HCO_3^- to CO_2 using CA (Raven et al. 1985; Finlay 2003).

Karst-associated nutrient enrichment and primary productivity

Karst hydrologic processes can influence primary producer communities by indirectly increasing the concentrations of limiting nutrients, including nitrogen and phosphorous, through rapid movement in subsurface flow paths (Notestein et al. 2003). This leads to higher nutrient content in groundwater and in groundwater-fed water bodies due to the more direct surface and subsurface connections (Leland & Porter 2000).

Nitrogen and phosphorous levels in rivers often influence primary productivity and accrual of primary producers (Lohman et al.1992; Dodds 2002). The effects of added nutrients largely depend on the surplus or shortage of nutrients already present. Primary

producers require fixed proportions of nitrogen and phosphorous (Redfield 1958). In the absence of other growth-limiting factors, adding limiting nutrients causes increases in algal growth (Francoeur 2001; Dodds et al. 2002). Most algae grow best with a 16:1 nitrogen-phosphorous ratio (Redfield 1958; Hillebrand & Sommer 1999). If the nitrogen to phosphorous ratio in water is greater than 20, the system is considered phosphorous limited, and if the nitrogen to phosphorous ratio is less than 10, the system is considered nitrogen limited (Busse et al. 2006). Nitrogen to phosphorous ratios between 10 and 20 indicate that nitrogen and phosphorous are both limiting nutrients. Adding nutrients already in surplus into a system, however, does not significantly affect algal growth (Francoeur 2001). Rivers can be nitrogen or phosphorous limited, or both nitrogen and phosphorous co-limited (Dodds et al. 2002). In some systems, other factors such as light limitation may limit primary productivity (Hill et al. 1995; Vanni et al. 2006). Therefore, while nutrient inputs related to karst hydrology are unlikely to affect primary producers in light limited systems, these inputs may impact algal and macrophyte abundances in nutrient-limited streams.

Primary productivity and midreaches

In-stream primary producers are more likely to be nutrient limited rather than light limited in midreaches of riverine systems. Midreaches are generally autotrophic, meaning that productivity exceeds respiration (Vannote et al. 1980; Rosi-Marshall & Wallace 2002) because they generally have an open canopy and shallow water, which allows sunlight to reach a large portion of the water column and some of the river bottom.

Streams of this size are generally characterized by high levels of algal growth and low retention rates for leaf detritus (Bott et al. 1985; Webster et al. 1994; Finlay 2001)

Other factors, such as dams and water velocity, can also modify stream water content and primary productivity. Dams can alter stream continuums by trapping particulate matter, which decreases inputs of organic matter from upstream and may also increase the clarity of water downstream (Ward & Stanford 1983). Processes that occur in a lake may also influence the composition of stream water downstream of the dam. Kling et al. (2000) found that lake processes tended to consume DIC, Ca^{+2}, Mg^{+2}, CO_2, CH_4, and NO_3^-, but outputs from lakes had increased K^+ and dissolved organic matter. Dams can also decrease DIC concentrations within the reservoir and in nearby downstream reaches. Prolonged residence time of the water column allows degassing until CO_2 concentrations within the water reach equilibrium with the atmosphere (Yang et al. 1996; Barth & Veizer 1999; Aucour et al. 1999). While dams may have some impact on downstream waters, this influence decreases with increasing distance from the dam (Ward & Stanford 1983).

Midreaches with open canopies are often colonized by several types of primary producers, namely vascular plants, mosses, cyanobacteria, diatoms, and green algae. Typically, attached algae dominate the primary productivity in midreaches (Lamberti & Steinman 1997; Rabeni & Sowa 2002). Macroalgae contribute greatly to primary production activity in many riverine systems (Power et al. 2009).The filamentous macroalgae *Cladophora* is present across a broad variety of marine and freshwater habitats around the globe (Dodds & Gudder 1992). *Cladophora* growth and accrual is positively correlated with increased nutrient supplies in rivers, and in some cases is

considered a nuisance species (Dodds & Gudder 1992; Penick et al. 2012). During warm weather in nutrient rich waters, *Cladophora* mats can grow so thick that they cover the entire river bottom (Penick et al. 2012).

Productivity and presence of this macroalgae, however, is temporary. *Cladophora* cover is also highly related to water velocity, and a recent study suggests that that *Cladophora* accrual may be more correlated to water velocity than nutrient levels in some streams (King et al. 2014). Riverine *Cladophora* growth often is highly seasonal during low-flow periods, and it is easily dislodged by high water events (Power 1990; Ensmingner et al. 2000; Power et al. 2009). Therefore, in areas where storm water pulses occur rapidly, such as in karst flow regimes, there may be enhanced conditions wherein *Cladophora* mats are dislodged, but few studies have addressed this possibility.

Primary consumers

Autochthonous food resources are an especially important component of herbivore diets in midreaches because algal based resources are typically more nutritious and more abundant than terrestrial-derived detritus (Vannote et al. 1980; Finlay 2001). Herbivores have lower C: N and C: P ratios than plant-based foods (Elser et al. 2000). While both algal and detrital food resources have fairly low nutritional value for consumers, autochthonous food resources are typically more nutritious because they have lower C: N ratios (Elser et al. 2000; Frost & Elser 2002; Lau et al. 2009). Growing evidence suggests that algal food resources are preferentially assimilated even in small or large streams where algal growth is limited (Frost & Elser 2002; Thorp & Delong 2002).

Although selective feeding can also influence a consumer's diet, increasing the abundance of a particular type of food generally increases the likelihood that it will contribute a higher proportion of carbon in the consumer's diet (Sterner & Hessen 1994; McNeely et al. 2007). Many taxa are generalist feeders and can switch food resources based on availability. Some studies have documented diet changes within a single taxa based on changes in season or stream order (Finlay 2001; Leberfinger et al. 2011).

Karst landscapes may be influential to an increase in nutrient levels and *Cladophora* standing stock during base flow (Penick et al. 2012). Differences in *Cladophora* standing stocks during low-water seasons, in turn, potentially affect stream food webs. *Cladophora* can provide a food supply to primary consumers either directly as a food resource or indirectly as a host to epiphytes. Although freshwater *Cladophora* is considered "grazer resistant," some macroinvertebrates use it directly as a food resource (Rhame & Stewart 1976). *Cladophora* can also be colonized by epiphytic microalgae (Peterson & Grimm 1992), particularly diatoms (Dodds 1991). Many of the epiphytes on *Cladophora* are labile food resources for consumers (Dodds 1991). *Cladophora*, epiphytes, and grazers can have complex beneficial interactions. Dodds (1991) found that epiphytes did not compete with *Cladophora* for nutrients and lowered drag on *Cladophora* tufts in reaches with high velocity, leading to reduced detachment and fragmentation. The epiphytes, however, also blocked photosynthesis under high density conditions. Primary consumers played a mediating role by removing up to 75% of epiphytes from *Cladophora* (Dodds 1991). Epiphyte assemblages also vary with water velocity and furthermore may be silica-limited during peak *Cladophora* growth (Bergey et al. 1995; Malkin et al. 2009).

Increasing *Cladophora* biomass may also affect niche breadth for an assemblage of primary consumers. Niche breadth is a measure of the range of different resources used by the consumer (MacArthur 1955; Layman et al. 2007). Consumers that are generalists tend to feed on foods according to availability (Feinsinger et al. 1981). Algal blooms may cause a few species of producers to be extremely abundant relative to other food resources, which may then cause consumers to rely increasingly on a few dominant species.

Stable isotopic analysis

Stable isotopic analysis is a common tool used to track the flow of nutrients and basal food resources in aquatic environments (Fry 2007; Hadwen & Bunn 2013; Stellato & Newman 2013). Stable isotopes, which do not undergo reactive decay, comprise less than ten percent of all known isotopes and are commonly found in nature in specific proportions (Fry 2007). Processes such as fractionation and mixing can change the ratio of the relative abundance of two isotopes of the same element within an environment or organism. During chemical reactions, one isotope (usually the "heavy" isotope) reacts marginally slower than the other ("light") isotope because of differences in atomic mass (Fry 2007). This process is called fractionation and it causes changes in the source processing of certain elements with unique signatures of ratios of "heavy" to "light" isotopes (Fry 2007).

Ratios of "heavy" to "light" isotopes are expressed in terms of permil, and values are calculated as higher ("enriched") or lower ("depleted") relative to a standard. The formula for calculating isotope values is $\delta X = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1{,}000$ (Ben-David &

Flaherty2012). The standard for $\delta^{13}C$ is Vienna Pee Dee Belemnite (VPDB) (Ben-David & Flaherty 2012). Since this source is largely composed of limestone, it is relatively $\delta^{13}C$-enriched relative to other sources. Most other sources of carbon are negative $\delta^{13}C$ relative to this source. The standard for $\delta^{15}N$ is atmospheric nitrogen (Ben-David & Flaherty 2012).

Carbon and nitrogen stable isotopes are commonly used to investigate both organic and inorganic carbon cycling in lotic environments (Fry 2007; Doctor et al. 2008; Schulte et al. 2011; McClanahan 2014). Carbon cycling is largely driven by exchanges of CO_2 in the atmosphere, soil, bedrock, and water. Carbon fractionation in primary producers occurs when plants preferentially use ^{12}C over ^{13}C during carbon fixation. Terrestrial plants with a C_3 photosynthetic pathway generally have fractionation around 20‰. Depending on the source of the CO_2, most terrestrial C_3 plants have carbon isotopic values between -34% to -22‰ (Finlay 2004). Stable carbon isotopes are much more variable in aquatic primary producers than terrestrial plants, notably because of the variation in $\delta^{13}C$ values of inorganic carbon, water velocity, and species of inorganic carbon available. Typically, primary producers in highly alkaline environments with low CO_2 conditions are expected to be relatively ^{13}C-enriched since HCO^- is ^{13}C-enriched relative to CO_2. Conversely, primary producers using CO_2 produced by respiration or living in reaches with high water velocity may have $\delta^{13}C$ values as low as -45‰ (Finlay 2003).

Surface streams with high groundwater inputs in limestone karst areas often have DIC that is more ^{13}C-enriched due to dissolved limestone. Limestone generally has a $\delta^{13}C$ value around 0‰, which is relatively ^{13}C-enriched compared to most other sources, and

dissolution from carbonates is typically a large contributing factor to overall DIC levels in karst streams (Schulte et al. 2011; Zavadlav et al. 2013). Primary producers in karst areas may have elevated $\delta^{13}C$ values compared with the same taxa in non-karst environments for two interrelated reasons: limestone contributions raises the $\delta^{13}C$ value of river water, and HCO_3^-, which is the dominant form of inorganic carbon in alkaline waters, is ^{13}C-enriched relative to CO_2 (Finlay 2004). Taxonomic differences, though, may be the main factor determining algal carbon isotope ratios in karst environments (Wang et al. 2013).

Nitrogen fractionation by primary producers is still largely unstudied. The $\delta^{15}N$ values of plants and algae are greatly influenced by a number of different enzymatic reactions as well as sources of nitrogen and growth rate (Fry 2007). The amount of nitrogen available has the potential to influence primary producer $\delta^{15}N$ values, especially when growth is limited by this nutrient (Woodland et al. 2012). Increased water velocity is also correlated with ^{15}N-depleted values in periphyton (Finlay et al. 2002; Rasmussen & Trudeau 2007).

Carbon and nitrogen isotopes are commonly used in trophic-dynamic and niche breadth studies. Consumers typically have similar $\delta^{13}C$ values compared to their food resources since little fractionation occurs during digestion and assimilation of carbon (Fry 2007). Studies have estimated that consumers have carbon isotope ratios that are about 0.5‰ enriched relative to their food resources (Fry 2007). Since different food resources in an area often have unique $\delta^{13}C$ signatures, the potential food resources that have similar $\delta^{13}C$ values to that of primary consumers are most likely to be the resources assimilated by that organism.

The addition of $\delta^{15}N$ values in trophic-dynamic studies is helpful where multiple potential food resources are present or when the trophic level of an organism is studied. Consumers at higher trophic levels are more ^{15}N-enriched relative to consumers at lower trophic levels. Invertebrate primary consumers typically have $\delta^{15}N$ values that are ^{15}N-enriched by 3.4‰ relative to their food resources.

Isotopes can be used to track changes in consumer diets that occur, such as longitudinal shifts in communities where conditions differ between upstream and downstream reaches (Phillips & Eldridge 2006). Shifts in stable isotope values are often associated with changes in consumer diets (Phillips & Eldridge 2006; Del Rio et al. 2009). These shifts alone, however, cannot be used as evidence of changes in consumer diets if spatial gradients correspond with similar isotopic patterns in food resources. Consumer isotopes will have similar isotopic spatial gradients as their food resources (Finlay 2001; McCutcheon & Lewis). In-stream primary producers in particular can have wide ranges of isotopic values, particularly $\delta^{13}C$ values (Fry 2007). The $\delta^{13}C$ values of in-stream primary producers are often influenced by both $\delta^{13}C$ values and availability of DIC (Finlay et al. 1999; Finlay 2001). The availability of DIC influences primary producer $\delta^{13}C$ values by increasing selectivity of ^{12}C during photosynthesis. Longitudinal gradients are common in algal resources. Specifically, Finlay (2001) found that algae often become increasingly ^{13}C-enriched downstream because of lower DIC abundances.

Mixing models are a commonly used in trophic-dynamic studies where consumers may have multiple food resources (Phillips & Gregg 2003). If consumers potentially assimilate numerous food resources, a definitive source contribution cannot be calculated (Fry 2007). Instead, mixing models can calculate a range of potential resource

contributions. The program IsoSource is a freeware program that calculates the range of feasible resource contributions for each potential food resource (Phillips & Gregg 2003).

Study area

The Green River Basin lies within the Interior Plateau and Interior River Valley and Hills Level III Ecoregions (Woods et al. 2002) and is the largest of Kentucky's twelve primary river basins, draining approximately 23,000 km^2 and nearly 23% of the Commonwealth's landscape. The Green River originates in Lincoln County, Kentucky, and flows nearly 600 km westward into the Ohio River as a 6^{th} to7^{th}-order system. Within the study area the parent geology is comprised of Paleozoic-age sedimentary rocks, including limestone, shale, and siltstone (Woods et al. 2002). Four study reaches were chosen within two sections with differing levels of karstification (Figure 1). The upstream study reaches were 43 and 47 Km downstream of the Green River Dam (GRD). The geology underlying the Green River in the upstream section of the study area is composed of relatively impure limestones with overlying and interbedded less-soluble siliciclastic rocks, while geology in the two study reaches further downstream (130 and 155 Km downstream of the GRD) is composed of continuous carbonate rocks (Osterhoudt 2014). A concurrent study found that the downstream reaches of the Green River were more strongly influenced by carbonate mineral dissolution than further upstream, as measured by specific conductance and pH (Osterhoudt 2014). A prior study found longitudinal differences between nutrient levels and *Cladophora* cover (Penick et al. 2012). Downstream reaches were characterized by high levels of nitrate and soluble reactive phosphorous relative to upstream reaches. *Cladophora* cover was also

significantly higher in downstream reaches relative to upstream reaches. Despite correlations between nutrient levels and *Cladophora* standing stock, however, nutrient enrichment studies suggested that algal accrual was not limited by total nutrient levels.

Although *Cladophora* in freshwater environments is considered grazer resistant, stable isotope studies suggest that *Cladophora*, along with attached epiphytes, are utilized as a food resource by a wide range of primary consumers during peak *Cladophora* blooms (Tinsley 2012; Yates 2012). These studies suggested that *Cladophora* was a prominent resource contributor for some invertebrate taxa during base flow periods in the Green River.

The influence of karst geology on nutrient levels and primary production has already been suggested in some studies (Leland & Porter 2000; Penick et al. 2012). This study sought to address how the availability of *Cladophora*, which is closely related to increasing karstification, influenced the diets of several species of primary consumers. This study addressed the following four questions in two hydrologically different years:

1. Do longitudinal trends in algal and consumer δ13C values relate to decreased DIC availability in larger watersheds?
2. Are trophic niche breadths narrower in more karstified reaches than in less karstified reaches due to longitudinal differences in *Cladophora* standing stocks?
3. Do differences in trophic-dynamic relationships between primary consumers and their food resources reflect the marked distinction in *Cladophora* standing stocks in two sections of the upper Green River that flow through differing levels of karstification?

4. Are consumers assimilating primarily autochthonous or allochthonous food resources?

CHAPTER 2: TROPHIC-DYNAMICS OF A TEMPERATE KARST RIVER DURING LATE SPRING/EARLY SUMMER

Introduction

Karst hydrology can influence primary productivity in two ways, by modifying DIC availability and contributing to increased nitrogen and phosphorous levels from surface runoff entering the groundwater supply and feeding surface streams from a spring (Raven et al. 1985; Leland & Porter 2000; Finlay 2003; Notestein et al. 2003; Schulte et al. 2011; Penick et al. 2012). Large scale factors such as drainage basin area and underlying geology, are important in determining long-term (>1 year) algal production, but micro scale factors can strongly influence short term algal production . Seasonal weather patterns, in particular, may cause predictable micro scale temporal patterns within consumer communities and food resource availability (Winemiller 1990; Townsend & Padovan 2005).

Seasonal variation in community structure and food webs is common (Winemiller 1990; Closs & Lake 1994). Fluctuations in discharge rates associated with seasonality especially can influence the relative abundance of food resources. Flooding brings in terrestrial particulate organic matter from outside the stream, which may be especially important for larger rivers with predictable flood cycles (Junk et al. 1989). Additionally, autumn leaf fall can bring a seasonal load of allochthonous food sources into streams in temperate climates (Benfield 1997). This food source, especially leaves with a slower breakdown rate, may be important in consumer diets during winter (Foucreau et al. 2013).

Algal standing stocks can also be mediated by seasonal changes in temperature and hydrological variability. Seasonal rainfall patterns influences both nutrient levels and algal growth (Finlay & Kendall 2007). During winter, however, primary production is likely to be limited by factors other than nutrient levels. Francoeur et al. (1999) found that periphyton levels were lower in winter and responded less to nutrient amendments than communities in summer, probably because of temperature differences. Peak flow events may also directly impact algal standing stocks. Periods with lower water velocity and less frequent hydrological disturbances have been associated with higher levels of algal accrual in some streams (Robinson & Minshall 1986; Townsend & Padovan 2005).

Macroalgae, in particular, are susceptible to scouring. Unlike some macrophytes (e.g., *P. ceratophyllum*) macroalgae lack holdfast or root structures and can be easily dislodged under high flow conditions (Power & Stewart 1987; Dodds & Gudder 1992). A study of a tropical river in Australia found that the macroalgae *Spirogyra* appeared during low flow periods and remained abundant until the first major high-water event in the fall (Townsend & Padovan 2005). Other studies on *Cladophora* have noted similar cycles of growth and biomass accrual based on hydrological conditions (Penick et al. 2012; Cattaneo et al. 2013).

Algal food resources are generally more nutritious than detrital sources, but algae is often limited by temperature and variable discharge rates during winter seasons (Elser et al. 2000; Huryn et al. 2001; Frost & Elser 2002; Lau et al. 2009). Consequently, a few studies have suggested that primary consumers within the study area shift from a detrital to an algal based diet. Zooplankton in two lentic systems ate recalcitrant allochthonous food sources during winter, but most growth occurred during the summer when they

assimilate more autochthonous sources (Grey et al. 2001; Rautio et al. 2011). Similarly, other primary consumers appear to shift from a detrital to an algal based diet during periods of high primary productivity (Huryn et al. 2001).

This study was conducted between late spring and early summer in the upper Green River, Kentucky during base flow conditions. Hydrologic flow in the Green River is often variable during winter and early spring, followed by periods of more stable base flow during summer and autumn and is considered eutrophic under base flow conditions (Penick et al. 2012). Seasonal cycles of *Cladophora* growth are also closely linked with hydrologic variability within this river. *Cladophora* grows most prolifically during base flow, but dislodges and becomes scarce during high water seasons.

While short-term, temporal factors facilitate algal growth during summer, reach-wide differences in *Cladophora* cover have been correlated with longitudinal changes within underlying geology (Penick et al. 2012). Other studies within higher *Cladophora* reaches have shown that consumers assimilate this type of algae as a food source (Tinsley 2012; Yates 2012). This study investigated whether the availability of *Cladophora*, which is closely correlated with increasing karstification, influenced the diets of several species of primary consumers. The following four questions were addressed during summer 2012:

1. Do longitudinal trends in algal and consumer δ13C values relate to decreased DIC availability in larger watersheds?
2. Are trophic niche breadths narrower in more karstified reaches than in less karstified reaches due to longitudinal differences in *Cladophora* standing stocks?

3. Do differences in trophic-dynamic relationships between primary consumers and their food resources reflect the marked distinction in *Cladophora* standing stocks in two sections of the upper Green River that flow through differing levels of karstification?
4. Are consumers assimilating primarily autochthonous or allochthonous food resources?

Methods

Study reaches

This research occurred in four reaches positioned along the upper Green River between the Green River Lake and Mammoth Cave National Park, Kentucky, USA. The Green River originates in Lincoln County, Kentucky and flows ca. 600 km west before emptying into the Ohio River. The Green River Basin is the largest of Kentucky's twelve primary river basins, draining approximately 23,000 km^2 and nearly 23% of the commonwealth. The upper portion of the Green River flows through the Interior Low Plateau region. This region of the eastern U.S. has a landscape characterized by low-relief topography and contains one of the most well-developed karst systems in the U.S. (Fenneman 1938; Palmer & Palmer 2009). Soils in the region are derived from weathering of limestone bedrock and additional allogenic sediments from non-carbonate rocks from the surrounding region and land is predominantly used for agriculture with mixed mesophytic forest (Woods et al. 2002).

The upper Green River flows over surficial geology that transitions from siliciclastic-dominated landscape upstream to highly karstified carbonate lithology

downstream. The two upstream study reaches are 43 and 47 km downstream of the Green River Dam and lie within the upstream basin. This basin drains 1,919 km^2 over heterogeneous surficial lithologies, mainly Devonian shale (38%) and Mississippian limestones (51%). Other lithologies in this basin include Ordovician dolostones and Mississippian sandstones (Osterhoudt 2014). The two downstream study reaches (130 and 155 km downstream of Green River Dam, respectively) lie within the downstream basin wherein surficial landscape is dominated by Mississippian carbonates (77%) (Palmer & Palmer 2009). Relatively small areas also include siliciclastic bedrocks such as shale, sandstone, and siltstone (Osterhoudt 2014). The upstream basin is nested within the downstream basin, and they collectively drain 4,489 km^2 (Osterhoudt 2014). Surface stream density is low in the valleys of the downstream basin (Woods et al. 2002) (Figure 1).

The upper Green River is a 6^{th}- (upstream reaches) to 7^{th}-order (downstream reaches) stream with well-defined banks at all four study reaches. Each study reach is located in a shallow riffle comparable in channel shape and depth with gravel and cobble substrates. Riparian edges are forested predominantly with red elm (*Ulmus rubra* Muhl.), silver maple (*Acer saccharinum* L.), box elder (*Acer negundo* L.), and American sycamore (*Platanus occidentalis* L.). Leaf retention is low in the main channel with packs in spring and summer largely restricted to sides of the channel or trapped in branches in downed logs and snags. Benthic organic matter trapped in substrate also appears to be largely made of leaf and wood detritus, and may serve as a reservoir of decayed leaf material within the channel.

The upper Green River is a eutrophic system. Nitrogen to phosphorous ratios are >20 in these reaches (Penick et al. 2012). While these ratios mildly suggest that this river could be phosphorous-limited, results from nutrient enrichment studies suggest that both nitrogen and phosphorous concentrations are higher than adequate to support growth (Grimm and Fisher 1986; Busse et al. 2006; Penick et al. 2012).

Macrophytes and macroalgae are present in shallow runs and riffles. Large patches of the riverweed *Podostemum ceratophyllum* (Michx.) were present in all four reaches. This macrophyte typically grows in fast flowing water and provides stable habitat for macroinvertebrates (Hutchens et al. 2004). *Cladophora* abundance is very low during winter and spring, but becomes increasingly abundant between late spring and fall when water levels were low (Penick et al. 2012). Upstream reaches displayed low *Cladophora* growth, characterized by small patches of filaments that were typically less than 0.5 m length. Downstream reaches had markedly higher *Cladophora* cover, with large mats covering extensive portions of the channel by August. Bryophytes and *Potamogeton* sp. were also present in the river channel.

The macroinvertebrate community is comprised mainly of a diverse assemblage of native mollusks plus a broad range of aquatic insect taxa. Numerous native freshwater mollusk species are found in the upper Green River, including federally-endangered species (Cicerello & Schuster 2003). Several unionids species in particular are abundant, namely *Actinonaias ligamentina* (Lamarck) (= mucket), *Amblema plicata* (Say) (= threeridge), *Cyclonaias tuberculata* (Rafinesque) (= purple wartyback), *Elliptio dilatata* (Rafinesque) (= spike), *Megalonaias nervosa* (Rafinesque) (= washboard), *Obliquaria reflexa* (Rafinesque) (= threehorn wartyback), *Quadrula quadrula* (Rafinesque) (=

mapleleaf), and *Tritogonia verrucosa* (Rafinesque) (= pistolgrip). The federally endangered *Cyprogenia stegaria* (Rafinesque) (= fanshell) and *Lampsilis abrupta* (Say) (= pink mucket) are present in adjacent reaches. The introduced Asiatic clam, *Corbicula fluminea* (Muller) is also abundant in all study reaches, as are at least three common freshwater snail species, especially *Leptoxis praerosa* (Say). Abundant aquatic insect taxa include ephemeropterans *Baetis* sp., *Caenis* sp., *Maccaffertium mediopunctatum* (McDunnough), *Serratella deficiens* (Morgan), and *Tricorythodes* sp., the giant stonefly *Pteronarcys dorsata* (Say), aquatic beetles *Dineutus* sp., *Stenelmis crenata* group, and *Psephenus herricki* (Dekay), the megalopteran *Corydalus cornutus* (L.), caddisflies *Cheumatopsyche* sp., *Hydropsyche simulans* (Ross), and *Oecetis* sp., and the dipteran blackfly *Simulium* sp. (Grubbs, unpublished data). Chironomid dipteran larvae are also very abundant but these have yet to be identified below the family level.

The Green River was at base flow for much of the study period between June and August 2012. The last high water event occurred during mid-May prior to the onset of this project (Figure 2). Discharge was consistently higher in the downstream reach. Mean discharge between May through October was 6315 L/s in the upstream reaches compared to 17415L/s in the downstream reaches. Differences in discharge rates reflect size differences in watersheds. The watershed in the two upstream reaches drains 1,191 Km^2 of the basin. The upstream watershed is nested within the downstream one, and collectively they drain 4,489 km^2 of the basin (Osterhoudt 2014). Water temperatures were slightly lower in the downstream reaches (Figure 3). Data were only collected from one upstream and downstream reach. Mean water temperature between May through October for the upstream reach was 24.3°C and 22.2°C in the downstream reach. Water

temperature in the upstream reaches were more influence by weather-related inputs while the downstream reaches were more strongly controlled by groundwater inputs due to a higher density of large springs (O'Driscoll & DeWalle 2006; Osterhoudt 2014).

Percent cover measurements

Cladophora and *Podostemum ceratophyllum* abundances in the four study reaches were quantified using a line transect method. Percent cover is a measure of the relative area covered by a particular species or group of interest and has been used in many terrestrial and aquatic settings (Brown 1975; Madsen 1999; Fiala et al. 2006). Percent cover of *Cladophora* and *P. ceratophyllum* was quantified along 10 randomly placed line transects within a 50-m long section at each study reach. Each transect was placed perpendicular to flow. Transects were 15 m long, except in the most upstream reach where the narrower river channel necessitated 10m transects.

Collection of food resources and consumers

This study generally followed the sampling procedures outlined in Delong and Thorp (2006) and Yates (2012). Food resources collected included leaves, *P. ceratophyllum*, *Cladophora*, epilithic biofilm, transported organic matter (TOM), and dissolved organic matter (DOM). Fresh green leaves of *A. negundo*, *A. saccharinum*, and *P. occidentalis* were collected from trees in the riparian zone and processed as three distinct samples. Although leaves were treated as a single food resource in a previous study (Delong & Thorp 2006), leaf species vary in nutritional quality to consumers (Sweeney et al. 1986). Previous research performed in this river suggests that stable

isotopic values of these leaves change little as they decay, indicating that stable isotopic values in fresh leaf samples are representative of isotope values of decaying leaves (Grubbs, unpublished data). In the lab leaves were rinsed with deionized water in lab to remove any impurities and dried for at least 48 hours at 70°C. Large veins were removed from dried leaves before further processing.

Cladophora and *P. ceratophyllum* were collected from the river bottom. Tips of both primary producers were torn or cut off and placed in chilled river water until samples could be processed. In upstream study reaches where *Cladophora* patches were generally very small, often the whole mat was collected. Only live tissue was included in samples. Previous research in this river indicated that isotopic ratios of decaying *Cladophora* also changes little over time; hence, fresh *Cladophora* are likely representative of both fresh and dead *Cladophora* tissue found in the river (Grubbs, unpublished data). *Podostemum* and *Cladophora* were first rinsed thoroughly in deionized water to remove dirt and large impurities, inspected under a dissection scope (7-10x), and any remaining detritus and consumers were removed before drying for at least 48 hours at 70° C. Epiphytes were present in many of these samples and were not removed before further processing.

Transported organic matter and DOM samples were obtained by collecting 60L of river water from the mid-channel. In the lab different size particles of TOM were separated by a series of sieves and filters. Samples were rinsed through nested 1000- and 100-µm sieves to obtain coarse (CTOM) and fine (FTOM) fractions, respectively. The CTOM fraction was subsequently discarded since there was only negligible material obtained during each sampling period. The remaining water sample was then vacuum-

filtered through a 1-µm Gelman glass fiber filter (GFF) to obtain an ultrafine (UFTOM) fraction. Because seston levels were typically very low, the FTOM and UFTOM fractions were usually combined for a single composited TOM fraction. DOM was obtained by evaporating river water after it had passed through a 1-µm glass fiber filter. Filtered water was heated, though not boiled, to facilitate evaporation. Sulfuric acid was added to the DOM water samples until water reached a pH of 2.0 to discourage microbe growth during filtering and processing.

Epilithic biofilm was scrubbed off of rocks. Rocks with relatively little sediment were preferentially selected and scrubbed in a bucket of water with a tooth brush to dislodge and collect epilithon. Biofilm was then concentrated by vacuum-filtering samples through a 1-µm GFF. Both TOM and biofilm was partitioned into separate algal and detrital fractions using a colloidal silica separation technique (Hamilton & Lewis 1992). Filtered samples were placed in 30mL of a 70% Ludox solution and centrifuged at 1200 rpm for 15 min to separate algal and detrital fractions. Samples were then observed and centrifuged again if the separation was incomplete. The resulting algal and detrital fractions were placed into separate centrifuge tubes with 30 mL of 70% Ludox and centrifuged again at the same rpm for 15 min to complete the separation process. Samples were rinsed with ample deionized water a final time and filtered through a 1-µm GFF to separate the sample from the Ludox solution.

Macroinvertebrate consumers were handpicked off of various substrates, including *P. ceratophyllum, Cladophora,* wood, leaves, and rocks in the river channel. Consumers were transported to the lab alive and placed in aquaria for a minimum of 24 hrs to allow their gut to clear. This allows any undigested food to pass through their gut

and prevent the stable isotope results from including components of their diets that are not assimilated into body tissue. Consumers were euthanized after this time in hot water and separated by taxa.

All samples were placed in ceramic crucibles and into a drying oven at 70^0 C for at least 48 hours. Dried samples were pulverized to a fine powder using a Wig-L-Bug®, measured out into two separate sample sizes for food resources (4.5 mg) and consumers (1.5 mg), and placed into 5x9 mm tin capsules. Carbon and nitrogen stable isotopic ratios were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility, University of Davis, California, USA. Stable isotope ratios were calculated as $\delta^{13}C$ or $\delta^{15}N$ (per mil) = $([R_{sample}/R_{standard}]-1) * 1000$, where R is the ^{13}C:^{12}C (=$\delta^{13}C$) or ^{15}N:^{14}N (=$\delta^{15}N$) ratio. Pee Dee Belemnite and atmospheric nitrogen (AIR) were used as standards for carbon and nitrogen analysis, respectively. Machine error for $\delta^{13}C$ was 0.2‰, and machine error for $\delta^{15}N$ was 0.3‰.

Statistical analyses

Percent cover of *Cladophora* and *P. ceratophyllum* was compared between the four study reaches using a non-parametric ANOVA. This statistical technique used a residual randomization procedure (RRP) with 9999 randomized permutations to produce a null distribution, which is then compared with the observed result. The p-value is determined by computing the rank percentile of the observed result within the distribution. The p-value was considered significant if $\alpha \leq 0.05$.

Niche breadth was computed by calculating the $\delta^{13}C$ range of primary consumers collected at each reach (Layman et al. 2007). The $\delta^{13}C$ range is expected to be wider in environments with several food resources with unique $\delta^{13}C$ signatures, which allows primary consumers to have diverse trophic niches (Layman et al. 2007). Stable isotope data for primary consumers were compared to data from potential food resources collected at the same study reach using IsoSource software following procedures outlined in Phillips & Gregg (2003). Trophic enrichment factors of 0.4 for $\delta^{13}C$ and 3.4 for $\delta^{15}N$ were added to all food resources prior to analyses to account for fractionation rates during digestion and assimilation processes (Phillips & Gregg 2003). Increment and tolerance levels were initially set at 1% and 0.2% for each analysis, respectively. When consumer isotopic values fell outside of mixing polygons, tolerance levels were increased by increments of 0.1% until either IsoSource produced feasible results or the tolerance level reached 0.7%. Potential food resources included leaves, *Cladophora,* algal and detrital components of epilithic biofilm, and transported organic matter (TOM). Two IsoSource analyses were run for each consumer, with and without *P. ceratophyllum.* Analyses that included P. *ceratophyllum* as a potential food resource returned high source contributions for this food resource. Previous studies, however, have suggested that *P. ceratophyllum* is not directly eaten by macroinvertebrates (Hutchens et al. 2004; Tinsley 2012). A second set of analyses excluded *P. ceratophyllum* in order to prevent spurious positive results for this food resource. IsoSource results were displayed as ranges of resource contributions (1-99th %tiles of all feasible results).

Results

Increasing karstification and longitudinal gradients in $\delta^{13}C$ values

Mean primary consumer, *Cladophora,* and *P. ceratophyllum* $\delta^{13}C$ values became increasingly ^{13}C-depleted in downstream reaches (Tables 1–2). These longitudinal gradients within $\delta^{13}C$ values of consumers and some resources were opposite to patterns found by Finlay (2001). While changes in consumer and in-stream producer $\delta^{13}C$ values cannot be clearly traced to DIC gradients in the Green River, Finlay (2001) suggested that longitudinal changes in DIC abundances may often cause gradients in algal $\delta^{13}C$ values.

Percent cover measurements

Cladophora cover varied significantly between reaches during summer 2012 (F-value= 13.48, r^2= 0.536, p< 0.001). Downstream reaches had significantly more cover compared to upstream reaches (Figure 5). Percent *Cladophora* cover in the two upstream reaches was 0.7% (±0.4) and 6.1% (±0.1). In contrast, percent *Cladophora* cover in the two downstream reaches was 30.7% (±9.4) and 34.6% (±3.2). Strands of *Cladophora* in the downstream reaches sometimes exceeded 10m in length, and much of the riverbed was covered by thick beds by July. In contrast, upstream reaches had smaller *Cladophora* mats that rarely grew >1m.

Podostemum ceratophyllum cover also varied significantly between individual study reaches (F-value=7.70, r^2=0.398, p<0.001). However, significant differences reflected inter-reach variation rather than longitudinal trends (Figure 4). *Podostemum ceratophyllum* cover in upstream reaches was not significantly different than downstream reaches. This macrophyte was visually abundant in all study reaches, growing in large

patches. Percent *P. ceratophyllum* cover was 43.2% (±6.7) and 21.5% (±1.7) in the two upstream reaches, and 13.7% (±4.1) and 36.2% (±5.6) in the downstream reaches.

Longitudinal gradients in niche breadth:

Longitudinal gradients occurred in consumer niche breadth, but these gradients do not provide evidence that this is caused by high abundances of *Cladophora* in downstream reaches. Niche breadth became slightly narrower in downstream reaches (Table 3). While this appears to correlate with increasing *Cladophora* standing stock (Figure 5), longitudinal gradients in niche breadth measurements cannot be attributed to the abundance of *Cladophora* downstream given that mixing models did not suggest that consumers assimilated more *Cladophora* downstream.

Longitudinal gradients in Cladophora assimilation

Isotopic longitudinal trends:

Longitudinal gradients in consumer isotopes did not provide clear evidence of a longitudinal shift in diet. Mean consumer $\delta^{13}C$ values become more ^{13}C-depleted downstream (Table 3). These longitudinal gradients, however, coincided with similar longitudinal gradients in multiple food resources. *Cladophora* and *P. ceratophyllum* also became more ^{13}C-depleted in downstream reaches, as did algal and detrital components of epilithic biofilm (Figure 6). Longitudinal gradients for epilithic biofilm are less certain, however, since samples of this food resource were not replicated.

Longitudinal trends in IsoSource results:

The mixing model analyses from IsoSource did not indicate that consumers assimilated less *Cladophora* in upstream reaches than downstream reaches. Analyses that included *P. ceratophyllum* typically had wide ranges of feasible solutions. Minimum *Cladophora* contribution was 0% for all samples (Figures 8–20).Maximum feasible contributions were as high as 78% (Figure 13), but maximum contributions were generally much lower in most samples (Figures 8–20). Median *Cladophora* contribution was <25% in all samples. Although median feasible contributions for *Cladophora* were slightly higher downstream for a few taxa (Figures 8, 9, 17, 18), most taxa displayed no gradient or had increased *Cladophora* assimilation downstream (Figure 10–14, 20). When *P. ceratophyllum* was excluded from mixing models, feasible solutions for *Cladophora* was typically high in all reaches (Figures 21-30). The only taxa that appeared to display an increase in *Cladophora* assimilation downstream was *P. herricki* (Figure 26). Results for other taxa either lacked solutions in both an upstream and downstream reach or suggested that *Cladophora* assimilation was not greater downstream (Figures 21–25, 26–30).

Consumer diets: autochthonous vs. allochthonous resources

Longitudinal gradients in consumer and resource isotopes

Longitudinal gradients in consumer $\delta^{13}C$ values tracked that of algal resources, providing partial evidence that consumers assimilated at least one autochthonous food resource. Overall, mean $\delta^{13}C$ values of primary consumers became more depleted in downstream reaches (Figure 6). *Cladophora* and *P. ceratophyllum* also became more ^{13}C-depleted in downstream reaches (Table 1). Both algal and detrital components of epilithic

biofilm also appeared to become more ^{13}C-depleted downstream, but this trend is uncertain due to a lack of replication at each reach. Other food resources, such as transported organic matter and terrestrial leaves were not more ^{13}C–depleted in downstream reaches.

Longitudinal gradients in consumer $\delta^{15}N$ values also tracked gradients found in some food resources, but these trends were too slight to provide clear evidence for consumption of any food resource. Mean consumer isotopes became slightly ^{15}N-depleted downstream (Figure 7). These trends matched gradients in $\delta^{15}N$ values of *Cladophora* (Table 1). Both algal and detrital components of epilithic biofilm and transported organic matter also appeared to have more ^{15}N-depleted values downstream, but these samples were not replicated. Tree leaves, alternatively, had increasingly-enriched $\delta^{15}N$ values from upstream to downstream. The $\delta^{15}N$ values of individual consumer taxa varied considerably, however, so no clear conclusions can be made (Figure 7).

IsoSource results

Results from both sets of mixing models suggested that primary consumers assimilated both autochthonous and allochthonous food resources. Different sets of mixing model analyses, however, differed in which autochthonous food resource was prominent. IsoSource mixing models that included *P. ceratophyllum* as a food resource generally indicated that most consumers had higher resource contributions for *P. ceratophyllum* and tree leaves relative to other food resources (Figures 8–20). *Podostemum ceratophyllum,* however, is not usually directly grazed by consumers

(Hutchens et al. 2004; Tinsley 2012). When *P. ceratophyllum* was excluded to avoid spurious false positive results, fewer analyses produced feasible solutions. Those analyses that returned feasible results suggested that consumers largely assimilate both *Cladophora* and tree leaves (Figures 21-30). Overall, mixing models that did not return feasible solutions had consumers that were more ^{13}C-depleted than food resources.

IsoSource Results: Reach A

IsoSource produced feasible results for eleven taxa in the furthest upstream reach when *P. ceratophyllum* was included as a food resource. Data from *Lepidostoma* and *P. dorsata* did not produce feasible solutions because they were markedly more ^{13}C-depleted than the potential food resources (Figures 35, 39). Models that produced feasible results indicated that consumer taxa had diets with relatively high ranges (1-99th percentiles) of potential resource contribution from *P. ceratophyllum* and tree leaves. Ten of the 11 taxa with feasible solutions had ranges with lower ends (1st percentile) >0% for *P. ceratophyllum*, suggesting that consumers assimilated at least some of this food resource (Figures 8–11, 13–15, 18–20). Six out of the 11consumer taxa had lower ranges (1st percentile) of feasible contributions for tree leaves >0% (Figures 8–9, 14–15, 19–20).

Only one of the thirteen consumer taxa collected at the furthest upstream reach produced feasible solutions when *P. ceratophyllum* was excluded from IsoSource models. *Hydropsyche* had high ranges (1-99th percentiles) for both *Cladophora* and tree leaves (Figure 22). This taxon was the most ^{13}C-enriched consumer (Table 1). Other consumers likely did not produce feasible solutions when *P. ceratophyllum* was excluded because they were more ^{13}C-depleted than all the food resources except tree leaves, although they

were more ^{15}N-enriched than tree leaves after accounting for trophic enrichment (Figures 31–32, 34–45).

IsoSource Results: Reach B

Twelve of the sixteen taxa from the second upstream reach produced feasible results in IsoSource models when *P. ceratophyllum* was included as a food resource. Out of the four taxa that had no feasible solutions, *P. dorsata, S. crenata,* and *Serratella* were outside mixing polygons because they were more ^{13}C-depleted than food resources (Figure 39, 44–45). The taxa that had feasible IsoSource solutions typically had high ranges (1-99th percentile) for *P. ceratophyllum* and tree leaves with one exception (Figures 8, 10–15, 18–19). *Psesphenus herricki* had low ranges for all food resources except for TOM (Figure17).

Seven consumer taxa had similar ranges for the food resource *Cladophora* and *P. ceratophyllum* (Figures 8, 10–12, 14, 17, 19). IsoSource ranges (1-99th percentile) for *Cladophora* were typically more variable than for *P. ceratophyllum.* These two food resources had similar carbon and nitrogen isotope values at this reach.

When *P. ceratophyllum* was excluded from the IsoSource models, nine of the sixteen consumer taxa produced feasible solutions. With only one exception, IsoSource results for these taxa indicated that consumers were assimilating *Cladophora* and tree leaves (Figures 21–25, 28–29). The IsoSource analysis also suggested that transported organic matter was the prominent food resource for *P. herricki* (Figure 26).

IsoSource Results: Reach C

Twelve of the thirteen taxa sampled in the first downstream reach produced feasible IsoSource solutions. Ranges for feasible source contributions were typically wider for all food resources than for results in the upstream reaches, although IsoSource results displayed more variation between taxa. Results for three taxa showed no prominent food resource (Figures 17, 18, 20). Results from other taxa suggested that leaves (Figures 11–14, 16) or *P. ceratophyllum* were prominent food resources (Figures 8–11, 13, 16). *Cladophora* carbon and nitrogen stable isotope values were similar to that of *P. ceratophyllum*, and some IsoSource results also indicated that the median resource contributions for *Cladophora* were comparable to that of *P. ceratophyllum* in five taxa (Figures 12, 14, 17–18, 20).

IsoSource models that excluded *P. ceratophyllum* at the first downstream reach produced feasible solutions for eight consumer taxa. Results suggested that leaves were a prominent contributor for seven taxa (Figures 21, 23–27, 30), and *Cladophora* appeared to be an important contributor for four taxa (Figures 21, 26, 27, 30). Analyses for all consumer taxa except *Maccaffertium* suggested that other food resources had low contributions (Figures 21, 23–24, 26–27, 30).

IsoSource Results: Reach D

Nine out of the eleven taxa at the furthest downstream reach produced feasible IsoSource solutions. *Optioservus* and *Simulium* that did not produce feasible solutions were more ^{15}N-depleted than all the food resources collected (Figures 38, 42). *Simulium* was also more ^{13}C-depleted than food resources (Figure 42). IsoSource results for this reach displayed the most variation between individual taxa. Consumers also displayed a

wider niche breadth than in the other three reaches (Table 7). While *P. ceratophyllum* and tree leaves appear to be important food resources for many taxa, other food resources, such as TOM and detrital epilithon, also appear to be additional important resource contributions for some taxa. Results for the consumer *Hydropsyche* indicate that *Podostemum* (33–37%) and detrital epilithon (51–66%) were the most prominent resource contributors (Figure 10). IsoSource results suggest that *Cladophora* (0–64%), *Podostemum* (0–29%), TOM (0–55%), and tree leaves (1–79%) were each potentially important food resources for *Isonychia* (Figure 11). Results for *Leptoxis* were less clear and had four potential major food resources: *Cladophora* (0–78%), *Podostemum* (10–43%), TOM (0–40%), and leaves (1–65%) (Figure 13). Results for *Maccaffertium* suggested that this consumer largely assimilated TOM (45–76%), but *Cladophora* (0–27%) and leaves (3–51%) were also be additional resource contributions (Figure 14). Mixing model solutions for *P. dorsata* had two clear dominant resource contributors, *Podostemum* (35–40%) and leaves (52–62%) (Figure 16). IsoSource models for *Pycnopsyche* resulted in three potential prominent resource contributors: *Cladophora* (0-54%), TOM (2-61%), and mean leaves (1-82%) (Figure 18). IsoSource results for *S. crenata* indicated that leaves were the main resource contributor (55–96%), but dietary contributions for *Cladophora* (0–24%) and TOM (2–61%) may also be additional food resources (Figure 20). Only four out of the eleven taxa produced feasible solutions when *P. ceratophyllum* was excluded from the mixing models. These four taxa had high source ranges for leaves (Figures 23, 25, 27, 30). Tree leaves were the only food resource that had high resource contributions for *Isonychia* (90–100%), *Pycnopsyche* (81–99%), and *S.*

crenata (95–100%) (Figures 23, 27, 30). IsoSource results for *Maccaffertium* suggested that this consumer assimilated leaves (50–62%) and TOM (32–46%) (Figure 25).

Mixing polygons

Consumers often had $\delta^{13}C$ values that were more depleted than most of the resources except for *P. ceratophyllum* and *Cladophora* (Figures 31–45). A number of consumers were more ^{13}C-depleted than all sampled resource values (Figures 32, 35, 38, 39, 42, 44). Consumer $\delta^{15}N$ values were often intermediate between relatively ^{15}N-enriched resources such as *P. ceratophyllum* and ^{15}N-depleted resources such as terrestrial leaves when trophic enrichment factors were included.

Consumer and resource $\delta^{13}C$ values

Food resources collected at the furthest upstream reach had $\delta^{13}C$ values that ranged between −29.92‰ ±0.4 (composite tree leaves) and −18.53‰ ±0.04 (*Cladophora*) (Table 1).The $\delta^{13}C$ values for primary consumers ranged from −32.32‰ (*P. dorsata*) to −24.04‰ (*H. simulans*) (Table 2). Food resources at the second upstream reach had $\delta^{13}C$ that ranged from −30.71‰ ±0.3 (composite tree leaves) to −15.91‰ (epilithic detritus). The $\delta^{13}C$ values for primary consumers ranged from −32.50‰ (*Serratella*) to −24.38‰ (*P. herricki*). Food resources at the first downstream reach had $\delta^{13}C$ values that ranged between −35.00‰ ±0.04 (*P. ceratophyllum*) and −15.36‰ (epilithic detritus). Primary consumer $\delta^{13}C$ values ranged between −35.38‰ (*Simulium*) and −28.64‰ (*Pycnopsyche*). All thirteen primary consumer taxa collected were more $\delta^{13}C$-depleted than the mean $\delta^{13}C$ value of food resources. Resources in the furthest downstream reach

had $\delta^{13}C$ values that ranged between −38.81‰ (*P. ceratophyllum*) and −25.18‰ (detrital epilithon). Primary consumers had $\delta^{13}C$ values that ranged from −36.31‰ (*Simulium*) and −28.56‰ (*Maccaffertium*). Two of the ten taxa collected from this reach were more ^{13}C-depleted than that of the mean for food resources.

Consumer and resource $\delta^{15}N$ values

Food resources collected at the furthest upstream reach had $\delta^{15}N$ values that ranged between 3.40‰ (TOM) to 7.87‰±0.02 (*Cladophora*) (Table 1). Consumer $\delta^{15}N$ values ranged from 8.23‰ (*Maccaffertium*) to 10.23‰ (*C. fluminea*) (Table 2). Food resources at the second upstream reach had $\delta^{15}N$ values that ranged from 3.42‰ (TOM) to 7.29‰±0.19 (*P. ceratophyllum*). Consumer $\delta^{15}N$ values at this reach ranged from 6.31‰ (*P. herricki*) to 9.66‰ (*C. fluminea*). Food resources at the first downstream reach had $\delta^{15}N$ values that ranged between 2.16‰ (TOM) to 6.86‰± 0.04 (*P. ceratophyllum*), and consumers ranged between 6.73‰ (*Maccaffertium*) and 9.04‰ (*H. simulans*). Resource $\delta^{15}N$ values at the furthest downstream reach ranged from 4.84‰±0.09 (*Cladophora*) to 8.03‰ (*P. ceratophyllum*). Considering resource values in the other three reaches, TOM most likely would have been the most ^{15}N-depleted food resource had it been successfully sampled. Consumer $\delta^{15}N$ values in the furthest downstream reach ranged from 6.95‰ (*S. crenata* group) to 10.31‰ (*H. simulans*).

IsoSource results: Functional feeding group trends

Overall, IsoSource analyses that included *P. ceratophyllum* indicated that the three functional feeding groups represented in this sample set did not assimilate differing

amounts of *Cladophora.* All sixteen analyses for filter feeders resulted in a minimum of 0% contribution for *Cladophora* (Figures 8–11, 19). Maximum contributions for *Cladophora* were variable and ranged between 0% and 68%, but all medians were < 25%. All shredder taxa resulted in a minimum of 0% contribution from *Cladophora* (Figures 12, 16, 18). The maximum feasible solution for *Cladophora* ranged between 0% and 54%, and the median contribution was >20% for all samples. All 11 analyses from grazer taxa resulted in a 0% minimum *Cladophora* contribution (Figures 13–15, 17, 20). Maximum contributions ranged between 2 and 62%, and median contributions were < 23%.

When *P. ceratophyllum* was excluded from the IsoSource models, fewer samples produced feasible solutions. Out of the samples that had solutions, filter feeders appeared to assimilate more *Cladophora*. Six out of the eight filter feeder analyses had a minimum contribution for *Cladophora* that was >0% (Figures 21–23, 28). Five samples had median *Cladophora* contributions >50% (Figure 21–23, 28). Overall, three of the four filter feeder taxa appeared to assimilate more *Cladophora* than leaves (Figure 21–22, 28). Two of the four samples of shredders resulted in a minimum *Cladophora* contribution >0% (Figures 24, 27). Medians for all were $\leq$ 40%. Three out of eight samples of grazers produced minimum contributions for *Cladophora* that were >0% (Figures 25–26, 29–30).

Discussion

Increasing karstification and longitudinal gradients in $\delta^{13}C$ values

Observed trends in *Cladophora* and *P. ceratophyllum* $\delta^{13}C$ values were surprising. Algal $\delta^{13}C$ values can vary greatly, and longitudinal gradients in algal $\delta^{13}C$

values are common since conditions vary between reaches (France 1995; Finlay et al. 1999; Finlay 2001). One meta-analysis by Finlay (2001) concluded that algae became increasingly ^{13}C-enriched downstream. Algal $\delta^{13}C$ values of this study, however, showed the opposite trend.

Algal $\delta^{13}C$ values can be influenced by several factors, including growth rate, species, water velocity, and supply of DIC (Finlay et al. 1999; Finlay 2001; Finlay 2004; Singer et al. 2005). DIC can influence primary producer $\delta^{13}C$ values primarily in two ways: by the $\delta^{13}C$ values of DIC itself and the abundance of DIC that is available to primary producers. The $\delta^{13}C$ values of DIC in the Green River are unlikely to contribute to longitudinal patterns in producer and consumer $\delta^{13}C$ values. One study found that $\delta^{13}C$ values of DIC were similar in all reaches and averaged between −12 to −14‰, depending on the season (McClanahan 2014). This also was slightly surprising, given that downstream reaches had more homogenous carbonate lithology than upstream reaches (Osterhoudt 2014). Groundwater and surface streams in limestone karst regions are expected to have DIC with relatively ^{13}C-enriched values due to DIC from carbonate rock, since limestone usually has a $\delta^{13}C$ value around 0‰ (Clark & Fritz 1995; Dubois et al. 2010). Despite more homogenous carbonate lithology in downstream reaches, the largest source contributor of DIC was soil respiration in all reaches (McClanahan 2014).

While trends in primary producer and consumer $\delta^{13}C$ values in the Green River cannot be definitively traced to any one cause, longitudinal gradients in algal $\delta^{13}C$ values sometimes occur as a result of longitudinal gradients in DIC availability. The abundance of DIC influences primary producer $\delta^{13}C$ values by changing fractionation rates during photosynthesis. Differential fractionation occurs because ^{12}C is preferentially used during

photosynthesis (Fry 2007). Carbon limitation will decrease fractionation rates and result in more ^{13}C-enriched values. Conversely, increasing availability of DIC to autotrophs during photosynthesis increases fractionation since more ^{12}C is available for photosynthesis (Finlay 2001). Increased concentration of DIC or increased water velocity is correlated with more ^{13}C–depleted values in algae (Hemminga & Mateo 1996; Burkhardt et al. 1999; Finlay et al. 1999; Riebesell et al. 2000; Finlay 2001). Increased water velocity, however, only has strong effects on algal $\delta^{13}C$ values when algae are carbon limited (Finlay et al. 1999).

Finlay (2001), which included data from 70 study reaches and 26 published studies, revealed that algal $\delta^{13}C$ values increased with watershed area (Finlay 2001). Watershed area was used as a proxy for DIC abundance. The concentration of CO_2 is typically lower in downstream reaches relative to upstream (Lorah & Herman 1988; Dawson et al. 1995; Jones & Mulholland 1998b; Finlay et al. 1999). This was the suggested cause of algal and consumer longitudinal trends within these studies.

Most streams have decreasing DIC availability in downstream reaches, but some factors may either mitigate or even reverse this trend. Abundances of DIC may actually increase downstream in the Green River, which would likely lead to observed trends in producer and consumer $\delta^{13}C$ values. One potential factor that may have decreased DIC concentrations upstream was the Green River Dam. All study reaches were between 43 and 155 km downstream of the Green River Dam. Lakes and reservoirs consume DIC both through processes within the lake and by degassing due to long residence times, decreasing the amount of CO_2 downstream (Yang et al. 1996; Barth & Veizer 1999; Aucour et al. 1999; Kling et al. 2000). No data on DIC availability was collected

upstream of the Green River Dam, however, so effects of this impoundment on water composition are uncertain.

The abundances of DIC may also increase downstream if downstream reaches receive large inputs of DIC-rich water or where rivers transition from a siliciclastic to carbonate lithologies. In one riverine system, DIC abundances increased downstream due to large inputs of water from poorly-degassed tributaries (Flintrop et al. 1996). Similarly, a number of large karst springs add large inputs of water into downstream reaches of the Green River (Blair et al. 2012). Spring inputs often have large amounts of DIC (Rounick & James 1984). Dissolved inorganic carbon may also become more available downstream if the underlying lithologies transition from siliciclastic to carbonate bedrocks. While increasing discharge is typically related to decreases in DIC, Concentrations of DIC are expected to increase with increasing karstification (Telmer & Veizer 1999; Schulte et al. 2011; Florea 2013). The upper Green River transitions from a more heterogeneous lithology upstream watershed to an almost purely carbonate-dominated lithology in downstream reaches (Figure 1). During one study during winter 2013, DIC concentrations in one of the upstream reaches ranged from 30-95 (mg/L), and 60-140 (mg/L) in one of the downstream reach (Osterhoudt 2014). While these measurements were taken during a short term study in winter, these longitudinal trends are consistent with findings from other studies and may persist in other seasons. If these longitudinal gradients in DIC availability are consistent, then primary producer and consumer $\delta^{13}C$ values are likely influenced by these patterns.

Although no definitive explanation can be given for longitudinal gradients in algal and consumer $\delta^{13}C$ values, primary producers may have become more ^{13}C-depleted

downstream in response to increasing concentrations of DIC. Concentrations of DIC usually decrease downstream in most streams, but influences such as changes in lithology and large karst spring inputs may potential reverse typical longitudinal gradients. Further investigations into the role of karst lithology on DIC availability and algal carbon isotopic values may reveal exceptions to the trends published in Finlay (2001), especially in karst areas where groundwater is often DIC-rich.

Percent cover

Karst geology is often associated with higher nutrient inputs and high algal accrual, including *Cladophora* (Leland & Porter 2000; Notestein et al. 2003; Penick et al. 2012). *Cladophora* is common in high nutrient waters and can be an important food resource for macroinvertebrate consumers (Rhame & Stewart 1976; Dodds 1991). Results for the two hypotheses tested in this study: that consumer diet and niche breadth would reflect differences in *Cladophora* standing stocks, were inconclusive.

Cladophora cover was significantly higher downstream in the highly-karstified carbonate reaches relative to the more siliciclastic upstream reaches (Figure 5). This observation is consistent from a previous study (Penick et al. 2012). The causes for this trend are uncertain, but higher nutrient levels, particularly nitrate, were correlated with *Cladophora* standing stocks in these reaches (Penick et al. 2012). Differences in *Cladophora* accrual do not appear to be related to hydrology, water temperature, substrate characteristics, or macroinvertebrate assemblages. While *Cladophora* is susceptible to scouring, relatively stable discharge rates during the study period minimized this factor, especially in the upstream reaches (Power 1990; Ensmingner et al.

2000; Power et al. 2009) (Figure 2). Other studies indicate that *Cladophora* grows best in warm water (Whitton 1970; Dodds & Gudder 1992), but standing stocks were higher in the cooler downstream reaches (Figure 3). Substrate characteristics were similar for all reaches, with a gravel-cobble mix and dense beds of *P. ceratophyllum*. In the downstream reaches *Cladophora* was commonly intertwined with *P. ceratophyllum* as a substrate for attachment. *Podostemum ceratophyllum* standing stocks had inter-reach variation but had no longitudinal trend between upstream and downstream reaches.

Longitudinal gradients in niche breadth

Consumer niche breadth decreased slightly in downstream reaches (Table 3). Consumers that are generalists tend to feed on foods according to availability (Feinsinger et al. 1981). Algal blooms may cause a few species of producers to be extremely abundant relative to other food resources, which may then cause consumers to rely increasingly more on a few dominant species. *Cladophora* standing stocks increased in downstream reaches (Figure 5). While longitudinal gradients in niche breadth appear to correlate with increasing abundance of *Cladophora,* differences in niche breadth are too slight to provide evidence of changes in food web structure.

Longitudinal gradients in Cladophora assimilation

Longitudinal gradients in mean consumer $\delta^{13}C$ values reflected changes within algal stable isotopes rather than changes in diet. Mean isotopic signatures of upstream consumer communities became increasingly ^{13}C-depleted downstream (Table 3). The ranges of consumer $\delta^{13}C$ values greatly overlapped in the upstream two reaches but

appeared distinct from the downstream two reaches (Figure 6). Longitudinal gradients within stable isotopes, particularly in carbon isotopes, are often associated with diet changes (Phillips & Eldridge 2006; Del Rio et al. 2009). These longitudinal trends, however, were similar to that of autochthonous food resources (Tables 1-2). Changes in $\delta^{13}C$ values of consumers likely occurred because consumer isotopic values reflected longitudinal gradients in $\delta^{13}C$ values of food resources rather than change in feeding habits.

Despite greater *Cladophora* accrual downstream, stable isotopic analyses did not indicate increased *Cladophora* assimilation downstream. Results from IsoSource analyses further confirmed that consumer diets showed no apparent longitudinal change (Figures 8-20). These findings were unexpected and run counter to other studies on longitudinal gradients. Upstream to downstream shifts in food resource availability are common and well documented (Vannote et al. 1980; Minshall et al. 1983; Naiman et al. 1987; Webster et al. 1994; Benfield 1997). Primary consumer diets are expected to reflect these longitudinal gradients (Vannote et al. 1980; Plague et al. 1998). One study investigating longitudinal dietary gradients between a 5^{th} and 7^{th} order stream found a that consumers assimilated more leaf detritus in the smaller upstream reaches and more filamentous algae and diatoms in the larger stream in response to food availability (Rosi-Marshall & Wallace 2002).

While consumer carbon and nitrogen isotopic values did not reflect differences in algal standing stocks during late spring and early summer, consumer isotopes sampled during this year may still reflect diets during the pre-*Cladophora* bloom spring season. All stable isotope samples were collected during June, beginning at the onset of the

Cladophora bloom in downstream reaches. Algal stable isotopes change quicker than that of primary consumers, and, consequently, consumer isotopic values often lag behind during seasonal shifts (Fry & Arnold 1982; Hesslein et al. 1993; Cabana & Rasmussen 1996). Seasonal shifts in consumer diets are also common, and may have influenced consumer isotopes at the time of study (Wainright et al. 1993; Fisher et al. 2001; Munoz 2003). Other studies during later summer and autumn found higher resource contributions for *Cladophora* at downstream reaches of this river (Tinsley 2012; Yates 2012). Additional analyses are needed to determine whether consumer diets more closely reflect *Cladophora* standing stocks later in the growing season.

Consumer diets: autochthonous vs. allochthonous resources

Stable isotope values of consumers indicated that primary consumers in these reaches assimilated a combination of algal and detrital food resources (Figures 8-20). All consumers and resources were collected from un-shaded riffles in a 6^{th}–7^{th} order stream. Current literature emphasizes the importance of algae as a food resource for consumers in midreaches. The River Continuum Concept (RCC) predicted that midreaches were characterized by high levels of primary production and consumer diets high in autochthonous resources (Vannote et al. 1980). Other studies have confirmed the importance of algae to consumers in this type of stream reach (Rosi-Marshall & Wallace 2002; Leberfinger et al. 2011).

Longitudinal gradients in consumer isotopes

Longitudinal gradients in primary consumer carbon stable isotopes provided evidence for an autochthonous-based diet. Consumers that assimilate algal resources should display similar trends in $\delta^{13}C$ and $\delta^{15}N$ values as algae (Finlay 2001; McCutcheon & Lewis 2002). Between-reach differences in mean consumer $\delta^{15}N$ values were small, approaching 1-2‰ (Figure 6). Mean isotopic signatures of upstream communities, however, were more ^{13}C-enriched than consumers downstream (Table 3). In addition, the ranges of consumer $\delta^{13}C$ values greatly overlapped in the upstream two reaches but appeared distinct from the downstream two reaches (Figure 6). These longitudinal gradients found in consumer $\delta^{13}C$ values were similar to algal resources, which also became increasingly ^{13}C-depleted downstream (Table 2). Longitudinal gradients within terrestrial leaves, in contrast, displayed a slight positive shift and did not explain trends within consumer $\delta^{13}C$ values.

Specifically, consumer $\delta^{13}C$ values were much closer to *Cladophora* and *P. ceratophyllum*, which were the most ^{13}C-depleted resources (Figure 6). The algal fraction of epilithic biofilm also appeared to have a similar longitudinal gradient in $\delta^{13}C$ values but was much more ^{13}C-enriched than consumer. Given the similarity in both $\delta^{13}C$ values and in isotopic longitudinal gradients, consumers likely assimilate either *Cladophora* or *P. ceratophyllum.*

IsoSource results

Results from IsoSource analyses further suggest that consumer taxa assimilated either *P. ceratophyllum* or *Cladophora* at all reaches. IsoSource models that included *P. ceratophyllum* as a food resource suggested that this resource was a more prominent

resource contributor than *Cladophora* (Figures 8-20). *Podostemum ceratophyllum* was the most ^{13}C-depleted food resource in three of the four reaches, and appeared more similar to consumer $\delta^{13}C$ values (Figure 6). Source contributions for *Cladophora* typically were low to moderate for most consumer taxa regardless of reach.

Source contributions for *P. ceratophyllum* were surprising. Studies have found that *P. ceratophyllum* supports higher macroinvertebrate densities by providing habitat (Grubaugh et al. 1997; Hutchens et al. 2004). Despite its importance to consumers, *P. ceratophyllum* is considered a low quality food resource and not usually directly grazed by macroinvertebrates (Hutchens et al. 2004; Tinsley 2012). Although decaying *P. ceratophyllum* may be used as a food resource, this macrophyte undergoes rapid decay and is unlikely to be an abundant food resource in the Green River during spring months (Hill & Webster 1982a). *Podostemum ceratophyllum* may indirectly supply food for invertebrates by acting as a substrate for epiphytes (Hutchens et al. 2004).

When *P. ceratophyllum* was excluded from IsoSource analyses, fewer analyses had feasible results because several consumer taxa had more ^{13}C-depleted values than any of the potential food resources. *Podostemum ceratophyllum* was excluded from models because studies suggest that this resource is not typically directly grazed by consumers (Hutchens et al. 2004; Tinsley 2012). In addition, mixing model results have less resolution when food resource isotopic values overlap (Fry 2007; Winemiller et al. 2007). Carbon and nitrogen isotopic values for *P. ceratophyllum* were similar to *Cladophora* in some reaches. Mixing model results that have false positive results from *P. ceratophyllum* may also have artificially lowered estimates of *Cladophora* assimilation. While this second set of analyses removed potentially spurious results, the carbon

isotopic values of the remaining potential resources did not fully explain the $\delta^{13}C$ values of some primary consumers.

Analyses that produced feasible solutions suggested that *Cladophora* was a prominent food resource for most consumer taxa at all reaches (Figures 21-30). Similar to *P. ceratophyllum, Cladophora* supports high macroinvertebrate densities partly by providing habitat (Fernandez & Reid 2012). *Cladophora* is has fairly low nutritional value as a food resource (Patrick et al. 1983; Dodds & Gudder 1992). Nevertheless, it can be grazed directly by some consumers (Rhame & Stewart 1976; Marcus et al. 1978; Grey & Ward 1979; Dodds 1991; McShaffrey & McCafferty 1991; Dodds & Gudder 1992). Several studies suggest that *Cladophora* provides a more important role in invertebrate diets by providing a substrate for labile epiphytes (Dodds 1991; Roll et al. 2005; Furey et al. 2012). Furey et al (2012) found that midges grazed on epiphytic algae and that *Cladophora* fragments appeared to be accidentally ingested.

Neither set of IsoSource analyses is more revealing than the other. Both sets had some consumer taxa that produced no feasible solutions because consumers were more ^{13}C-depleted than any of the food resources sampled. While models that included *P. ceratophyllum* had a higher success rate at returning statistically feasible solutions, high resource contributions for live *P. ceratophyllum* seem unrealistic given the low quality of the food resource. Most published studies suggest that consumers do not directly consume this food resource (Hill & Webster 1984; Tinsley 2012). Alternatively, only about half of the IsoSource models excluding *P. ceratophyllum* produced statistically feasible solutions, often because some consumers had more ^{13}C-depleted values than any of the other food resources (Figures 31-45). Consumer $\delta^{13}C$ values that fall outside of the

range of sampled food resources has been used as evidence for a missing food resource (Winemiller et al. 2011). If this study failed to collect a crucial food resource, this missing food resource is likely to be algal-based rather than from terrestrial detritus. Consumer tissues had more ^{13}C-depleted values than terrestrial resources, and consumer trends within longitudinal gradients closely tracked that of algal resources. Alternatively, the ^{13}C-depleted values found in these consumers may still reflect algal isotopic values of food resources consumed during early spring.

Potential seasonal influences

As mentioned previously, algal $\delta^{13}C$ values undergo seasonal shifts and consumer stable isotopes may lag behind changes in food resources (Fry & Arnold 1982; Hesslein et al. 1993; Cabana & Rasmussen 1996). Algal $\delta^{13}C$ values in another study were more ^{13}C-depleted during high water spring seasons than during base flow conditions (McCutcheon & Lewis 2002). Although no early spring food resources have been sampled in the upper Green River, algal stable isotopic values may fluctuate between the higher-water spring period and the lower base flow conditions during the study period. Frequent peak flow events during winter and spring may have also brought an influx of dissolved organic matter from the Green River dam located upstream of the study reaches.

Consumer diets: autochthonous food resources

Longitudinal gradients in consumer and resource $\delta^{13}C$ values and mixing model results suggest that consumers partially rely on autochthonous resources. Consumers may

have assimilated *P. ceratophyllum*, *Cladophora,* associated epiphytes, or a combination of these resources. While results from the first set of IsoSource analyses suggests that consumers largely assimilate *P. ceratophyllum* (Figures 8-20), this is unlikely given that other studies have concluded that few consumers directly graze this resource (Hutchens et al. 2004; Tinsley 2012). Alternatively, the second set of IsoSource analyses resulted in less feasible solutions, but concluded that consumers assimilated largely *Cladophora* (Figures 21-30). This resource is sometimes directly grazed by consumers (Rhame & Stewart 1976). Consumers may have also consumed epiphytes, which were not isolated and directly sampled. Dense series of epiphytes were observed in the laboratory on strands of *Cladophora* and *P. ceratophyllum* under a dissecting microscope. While larger pieces of detritus and consumers were picked off with forceps, epiphytes were not removed from either *Cladophora* or *P. ceratophyllum*. Studies on consumer communities within *P. ceratophyllum* and *Cladophora* mats indicate that epiphytes may be a more important portion of macroinvertebrate diets than the macrophytes or macroalgae itself (Furey et al. 2012). This is plausible in the upper Green River. Observations of a chironomid feeding during this study showed that this consumer scraped diatom epiphytes off of *Cladophora* strands. A fragment of *Cladophora* was ingested during the observation and passed through the gut tract visibly unchanged (personal observation). Though no observations were made of other taxa feeding, some taxa likely assimilated more epiphytes than *Cladophora.*

Consumer diets: allochthonous food resources

In addition to strong evidence to support the importance of autochthonous food resources in this food web, terrestrial tree leaves also remained an important component of consumer diets. After taking trophic enrichment factors into account, consumers were consistently more ^{15}N-depleted than either *P. ceratophyllum* or *Cladophora* (Figures 31-45). This implied that they also assimilated an additional food resource. Leaves were more ^{15}N-depleted than consumer tissues (Figure 6). Both sets of IsoSource analyses indicated that consumer taxa assimilated tree leaves (Figures 8-30). Unconditioned tree leaves are a poor quality food resource compared to most algal resources (Barlocher & Kendrick 1975). However, tree leaves become a more labile resource for consumers after conditioning by bacteria and fungi (Barlocher & Kendrick 1975; Graca et al. 2001).

Consumer diets: summary

Consumers in midreaches are expected to rely less on leaves because this size stream typically has less leaf inputs and retention and higher abundances of autochthonous food resources (Vannote et al. 1980; Bott et al. 1985; Webster et al. 1994; Finlay 2001). In addition, recent studies in both headwater streams and large streams have emphasized the disproportionate importance of algal to macroinvertebrate diets even where detrital resources are more abundant (Mayer & Likens 1987; Delong & Thorp 2006; McNeely et al. 2007). These results, however, suggest that leaves continue to be an additional important food resource even where algal and macrophyte standing stocks are relatively high. This may be especially true during low-productivity seasons; Leaves that become conditioned at slower rates, in particular, may provide food for consumers during winter and early spring when algal growth is low (Foucreau et al. 2013).

Summary

Consumer and in-stream primary producer $\delta^{13}C$ values became more ^{13}C-depleted in downstream reaches. While the cause of this pattern cannot be definitively identified, longitudinal gradients in DIC availability have been linked with longitudinal trends in $\delta^{13}C$ values of algae in other streams (Finlay 2001). Observed trends in this study, however, were opposite to typical longitudinal trends in algal $\delta^{13}C$ values, suggesting that DIC availability may have increased in downstream reaches. Increasing karstification and large inputs from karst springs in downstream reaches may have reversed typical longitudinal trends in DIC availability, though future studies will be needed to confirm this explanation.

Carbon and nitrogen isotopic values of consumers in this study were likely influenced by diets during pre-*Cladophora* bloom conditions. Analyses suggested that primary consumers had similar diets and niche breadths in all reaches. Consumers also appeared to assimilate a mix of autochthonous and allochthonous resources. What remains unclear, however, is which in-stream primary producer(s) were assimilated by consumers. Consumers may assimilate *P. ceratophyllum, Cladophora,* associated epiphytes, or a combination of these resources. Although consumers are expected to rely largely on autochthonous resources in midreaches, mixing model analyses also suggested that tree leaves were a prominent food resource as well. These results suggest that consumers in midreaches may still supplement their diets with allochthonous resources, especially during periods with lower algal growth.

CHAPTER 3: TROPHIC-DYNAMICS OF A TEMPERATE RIVER DURING LATE SUMMER/ EARLY AUTUMN

Introduction

Karst-related hydrology can influence primary productivity by modifying DIC availability, and it is often correlated with increased nitrogen and phosphorous levels (Raven et al. 1985; Leland & Porter 2000; Finlay 2003; Notestein et al. 2003; Schulte et al. 2011; Penick et al. 2012). Large scale factors such as drainage basin and underlying geology are important factors determining long-term (>1 year) algal production, but micro-scale factors can strongly influence short term algal production (Biggs & Gerbeaux 1993; Snyder et al. 2002). Biggs & Gerbeaux (1993) found that long term periphyton growth was most strongly correlated with nutrient inputs associated with underlying marble bedrock, but short term periphyton growth was best explained by micro-scale factors.

Seasonality and hydrology can affect primary production, terrestrial subsidies, and, consequently, food web structure (Junk et al. 1989; Winemiller 1990; Townsend & Padovan 2005). Seasonal variation in community structure and food webs is common (Winemiller 1990; Closs & Lake 1994). Flooding brings in terrestrial particulate organic matter from outside the stream, which may be especially important for larger rivers with predictable flood cycles (Junk et al. 1989). Additionally, autumn leaf fall can bring a seasonal load of allochthonous food sources into streams in temperate climates (Vannote et al. 1980; Benfield 1997). This food resource, especially leaves with a slower

breakdown rate, may be important in consumer diets during winter (Foucreau et al. 2013).

Algal standing stocks can also be mediated by seasonal changes in temperature and hydrology. Seasonal rainfall patterns influences both nutrient levels and algal growth (Finlay & Kendall 2007). During winter, however, primary production is likely to be limited by factors other than nutrient levels. Francoeur et al. (1999) found that periphyton levels were lower in winter and responded less to nutrient amendments than communities in summer, probably because of temperature differences.

Peak flow events may also directly impact algal standing stocks. Periods with lower velocity and less frequent hydrological disturbances have been associated with higher levels of algal accrual (Robinson & Minshall 1986; Townsend & Padovan 2005). Conversely, frequent spates are correlated with lower algal biomass because subsequent movement of substrate and fine sediment causes scouring and light limitation (Yule 1996; Power et al. 2008; Yang et al. 2009). Macroalgae, in particular, are susceptible to scouring and can be easily dislodged under peak flow conditions (Power & Stewart 1987, Dodds & Gudder 1992). A study of a tropical river in Australia found that the macroalgae *Spirogyra* appeared during low flow periods and remained abundant until the first major high-water event in the fall (Townsend & Padovan 2005). Other studies on *Cladophora* have noted similar cycles of growth and biomass accrual based on hydrological conditions (Power et al. 2008; Penick et al. 2012; Cattaneo et al. 2013).

Algal food resources are generally more nutritious than detritus, but algae is often limited by temperature and hydrologic variability during winter seasons in some areas of the world (Elser et al. 2000; Huryn et al. 2001; Frost & Elser 2002; Lau et al. 2009).

Consequently, some primary consumers appear to shift from an algal to a detrital based diet during either winter months or periods with frequent spates. Zooplankton in two lentic systems ate recalcitrant allochthonous food sources during winter, but most growth occurred during the summer when they assimilated more autochthonous sources (Grey et al. 2001; Rautio et al. 2011). Other primary consumers also appeared to shift from a detrital to an algal based diet during periods of high primary productivity (Huryn et al. 2001). Similarly, spates can alter macroinvertebrate densities and food web structure. Yule (1996) found that frequent spates caused low algal levels and detritus-based food webs due to scouring and movement of substrate.

This study was conducted between late summer and early autumn 2013 in the upper Green River, Kentucky. Discharge in the Green River is usually highest during winter and early spring, followed by low water periods during summer and autumn (Penick et al. 2012). Frequent spates, however, occurred during this study period (Figure 2). Seasonal cycles of *Cladophora* growth are also closely linked with hydrologic variability in this river. *Cladophora* grows most prolifically during base flow, but dislodges and becomes scarce during high water seasons.

While short-term, temporal factors facilitate algal growth during summer, reach-wide differences in *Cladophora* cover have been correlated with longitudinal changes within underlying geology (Penick et al. 2012). Other studies within higher *Cladophora* reaches have shown that consumers assimilate this type of algae as a food source (Tinsley 2012, Yates 2012). This study addressed how the availability of *Cladophora*, which is closely correlated with increasing karstification, influenced the diets of several species of

primary consumers. This study addressed the following four questions during autumn 2013:

1. Do longitudinal trends in algal and consumer δ13C values relate to decreased DIC availability in larger watersheds?
2. Are trophic niche breadths narrower in more karstified reaches than in less karstified reaches due to longitudinal differences in *Cladophora* standing stocks?
3. Do differences in trophic-dynamic relationships between primary consumers and their food resources reflect the marked distinction in *Cladophora* standing stocks in two sections of the upper Green River that flow through differing levels of karstification?
4. Are consumers assimilating primarily autochthonous or allochthonous food resources?

Methods

Study reaches

This research occurred in four reaches positioned along the upper Green River between the Green River Lake and Mammoth Cave National Park, Kentucky, USA. The Green River originates in Lincoln County, Kentucky, and flows ca. 600 km west before emptying into the Ohio River. The Green River Basin is the largest of Kentucky's twelve primary river basins, draining approximately 23,000 km^2 and nearly 23% of the commonwealth. The upper portion of the Green River flows through the Interior Low Plateau region. This region in the eastern U.S. has a landscape characterized by low-relief

topography and contains one of the most well-developed karst system in the U.S. (Fenneman 1938; Palmer & Palmer 2009). Soils in this region are derived from weathering of limestone bedrock, and land is predominantly used for agriculture with mixed mesophytic forest (Woods et al. 2002).

The upper Green River flows over surficial geology which transitions from a more heterogeneous lithology upstream to a highly karstified carbonate lithology downstream. The two upstream study reaches are 43 and 47 km downstream of the Green River Lake and are within the upstream basin. This basin drains 1,919 km^2 over heterogeneous surficial lithologies, mainly Devonian shale (38%) and Mississippian limestones (51%). Other lithologies in this basin include Ordovician dolostones and Mississippian sandstones (Osterhoudt 2014). The downstream two study reaches (130 and 155 km downstream the Green River Lake) lie within the downstream basin whose surficial landscape is dominated by Mississippian carbonates (77%) (Palmer & Palmer 2009). Relatively small areas also include siliciclastic bedrocks such as shale , sandstone, and siltstone (Osterhoudt 2014). The upstream basin is nested within the downstream basin, and they collectively drain 4,489 km^2 (Osterhoudt 2014) (Figure 1). Surface stream density is low in the valleys of the downstream basin (Woods et al. 2002).

The upper Green River is a 6^{th}- (upstream reaches) to 7^{th}-order (downstream reaches) stream with well-defined banks at all four study reaches. Each study reach was located in a shallow riffle comparable in channel shape and depth with gravel and cobble substrates. Riparian edges are forested predominantly with red elm (*Ulmus rubra* Muhl.*)*. silver maple (*Acer saccharinum* L.*),* box elder (*Acer negundo* L.*),* and American sycamore (*Platanus occidentalis* L.*)*. Leaf retention is low in the main channel with packs

in spring and summer largely restricted to sides of the channel or trapped in branches in downed logs and snags. Benthic organic matter trapped in substrate also appears to be largely made of leaf and wood detritus, and may serve as a reservoir of decayed leaf material within the channel.

The upper Green River is a eutrophic system. Nitrogen to phosphorous ratios are >20 in these reaches (Penick et al. 2012). While these ratios mildly suggest that this river could be phosphorous-limited, results from nutrient enrichment studies suggest that both nitrogen and phosphorous concentrations are higher than adequate to support growth (Grimm and Fisher 1986; Busse et al.2006; Penick et al. 2012).

Macrophyte and macroalgae are present in shallow runs and riffles. Large patches of the riverweed *Podostemum ceratophyllum* (Michx.) were present in all four reaches. This macrophyte typically grows in fast flowing water and provides stable habitat for macroinvertebrates (Hutchens et al. 2004). *Cladophora* abundance is very low during winter and spring, but becomes increasingly abundant between late spring and fall when water levels were low (Penick et al. 2012). Upstream reaches displayed low *Cladophora* growth, characterized by small patches of filaments that were typically less than 0.5 m length. Downstream reaches had markedly higher *Cladophora* cover, with large mats covering extensive portions of the channel by August. Bryophytes and *Potamogeton* sp. are also present in the river channel.

The macroinvertebrate community is comprised mainly of a diverse assemblage of native mollusks plus a broad range of aquatic insect taxa. Numerous native freshwater mollusk species are found in the upper Green River, including federally-endangered species (Cicerello & Schuster 2003). Several unionids species in particular are abundant,

namely *Actinonaias ligamentina* (Lamarck) (= mucket), *Amblema plicata* (Say) (= threeridge), *Cyclonaias tuberculata* (Rafinesque) (= purple wartyback), *Elliptio dilatata* (Rafinesque) (= spike), *Megalonaias nervosa* (Rafinesque) (= washboard), *Obliquaria reflexa* (Rafinesque) (= threehorn wartyback), *Quadrula quadrula* (Rafinesque) (= mapleleaf), and *Tritogonia verrucosa* (Rafinesque) (= pistolgrip). The federally endangered *Cyprogenia stegaria* (Rafinesque) (= fanshell) and *Lampsilis abrupta* (Say) (= pink mucket) are present in adjacent reaches. The introduced Asiatic clam, *Corbicula fluminea* (Muller) is also abundant in all study reaches, as are at least three common freshwater snail species, especially *Leptoxis praerosa* (Say). Abundant aquatic insect taxa include ephemeropterans *Baetis* sp., *Caenis* sp., *Maccaffertium mediopunctatum* (McDunnough), *Serratella deficiens* (Morgan), and *Tricorythodes* sp., the giant stonefly *Pteronarcys dorsata* (Say), aquatic beetles *Dineutus* sp., *Stenelmis crenata* group, and *Psephenus herricki* (Dekay), the megalopteran *Corydalus cornutus* (L.), caddisflies *Cheumatopsyche* sp., *Hydropsyche simulans* (Ross), and *Oecetis* sp., and the dipteran blackfly *Simulium* sp. (Grubbs, unpublished data). Chironomid dipteran larvae are also very abundant but these have yet to be identified below the family level.

The Green River rose above base flow numerous times between May and October 2013 (Figure 2). Discharge was consistently higher in the downstream reach, most likely due to larger watershed area in the downstream reaches. Discharge was only measured at one upstream and downstream reach. Mean discharge between May through October was 26,616 L/s in the upstream reach and 71,581 L/s in the downstream reach. Water levels were too high to safely work at study reaches ca. 28,000 L/s, especially in the downstream reach during most of June-August 2013. Field work took place between July

and October when river levels were lower. High water events frequently scoured the river bed during the sampling period, and most new *Cladophora* growth was washed downstream after these events. Water temperatures were slightly lower in the downstream reach compared to the upstream reach (Figure 3). The mean water temperature between May through October for the upstream reach was 21.5°C and 20.9°C for the downstream reach. Water temperatures in both reaches were cooler during part of June and July than in May and August. This drop coincided with consistent elevated discharge levels and Green River Lake releases during this time. Water temperature in the upstream reach was more strongly controlled by weather-related inputs while the downstream reach was more strongly controlled by groundwater inputs due to a higher density of large springs (O'Driscoll & DeWalle, 2006; Osterhoudt 2014).

Percent cover measurements

Cladophora and *Podostemum ceratophyllum* abundances in the four study reaches were quantified using a line transect method. Percent cover is a measure of the relative area covered by a particular species or group of interest and has been used in many terrestrial and aquatic settings (Brown 1975; Madsen 1999; Fiala et al. 2006). Percent cover of *Cladophora* and *P. ceratophyllum* was quantified along 10 randomly placed line transects within a 50-m long reach at each study reach. Each transect was placed perpendicular to flow. Transects were 15 m long, except in the most upstream reach where the narrower river channel necessitated 10 m transects.

Collection of food resources and consumers

This study generally followed the sampling procedures outlined in Delong and Thorp (2006) and Yates (2012). Food resources collected included leaves, *P. ceratophyllum*, *Cladophora*, epilithic biofilm, wood biofilm, transported organic matter (TOM), and benthic organic matter (BOM). Fresh green leaves of *A. negundo*, *A. saccharinum*, and *P. occidentalis* were collected from trees in the riparian zone and processed as three distinct samples. Although leaves have been treated as a single food resource in a previous study (Delong & Thorp 2006), leaf species vary in nutritional quality to consumers (Sweeney et al. 1986). Previous research done in this river suggests that stable isotopic values of these leaves change little as they decay, indicating that stable isotopic values in fresh leaf samples are representative of isotope values of decaying leaves (Grubbs, unpublished data). In the lab leaves were rinsed with deionized water in lab to remove any impurities and dried for at least 48 hours at 70°C. Large veins were removed from dried leaves before further processing.

Cladophora and *P. ceratophyllum* were collected from the river bottom. Tips of both primary producers were torn or cut off and placed in chilled river water until samples could be processed. In upstream study reaches where *Cladophora* patches were generally very small, often the whole mat was collected. Only live tissue was included in samples. Previous research in this river indicated that isotopic ratios of decaying *Cladophora* also changes little over time, Hence, fresh *Cladophora* are likely representative of both fresh and dead *Cladophora* tissue found in the river (Grubbs, unpublished data). *Podostemum* and *Cladophora* were first rinsed thoroughly in deionized water to remove dirt and large impurities, inspected under a dissection scope (7-10x), and any remaining detritus and consumers were removed before drying for at

least 48 hours at 70°C. Epiphytes were present in many of these samples and were not removed before further processing.

Transported organic matter and DOM samples were obtained by collecting 120 L of river water from the mid-channel. In the lab different size particles of TOM were separated by a series of sieves and filters. Samples were rinsed through nested 1000- and 100-µm sieves to obtain coarse (CTOM) and fine (FTOM) fractions, respectively. The CTOM fraction was subsequently discarded since there was only negligible material obtained during each sampling period. The remaining water sample was then vacuum-filtered through a 1-µm Gelman glass fiber filter (GFF) to obtain an ultrafine (UFTOM) fraction. Because seston levels were typically very low, the FTOM and UFTOM fractions were usually combined for a single composited TOM fraction. DOM was obtained by evaporating river water after it had passed through a 1-µm glass fiber filter. Filtered water was heated, though not boiled, to facilitate evaporation. Sulfuric acid was added to the DOM water samples until water reached a pH of 2.0 to discourage microbe growth during filtering and processing.

Benthic organic matter was collected by piezometer constructed of PVC pipe and a plunger that applied suction. Surface benthic organic matter was collected by placing the bottom of the piezometer directly on the benthos and applying suction. After the surface BOM had been collected, the deep BOM samples were collected by placing the piezometer back into the area where the surface BOM had been removed and applying suction to obtain a core sample of sediment. In the lab, deep BOM was split into different size components using a series of sieves and filters. Deep BOM samples were swirled in their original containers until inorganic components settled to the bottom. The organic

portion of the water sample was poured over a series of sieves and separated into size fractions that were >1000µm, 1000-250- µm, and 250-100 µm. An ultrafine BOM fraction (100-1µm) was obtained by filtering the remaining sample through a 1-µm Gelman glass fiber filter (GFF). Surface BOM samples were swirled to settle inorganic sediments, and the organic portion was decanted off and filtered through a 1-µm glass fiber filter (GFF). All size fractions of surface BOM were treated as one composite sample.

Epilithic biofilm was scrubbed off of rocks. Rocks with relatively little sediment were preferentially selected and scrubbed in a bucket of water with a tooth brush to dislodge and collect epilithon. Wood biofilm was collected by carefully scraping biofilm off of the surface of wood using a scalpel or soft brush. Both types of biofilm were then concentrated by vacuum-filtering samples through a 1-µm GFF. Wood biofilm was separated into algal and detrital fractions using a colloidal silica separation technique (Hamilton & Lewis 1992). Filtered samples were placed in 30 mL of a 70% Ludox solution and centrifuged at 1200 rpm for 15 min to separate algal and detrital fractions. Samples were then observed and centrifuged again if the separation was incomplete. The resulting algal and detrital fractions were placed into separate centrifuge tubes with 30 mL of 70% Ludox and centrifuged again at the same rpm for 15 min to complete the separation process. Samples were filtered a final time with the 1-µm GFF to separate the sample from the Ludox solution. Samples were rinsed with ample deionized water a final time and filtered through a 1-µm GFF to separate the sample from the Ludox solution. Neither TOM nor epilithic biofilm were partitioned into separate algal and detrital

fractions. Attempts at separating the two fractions of these resources yielded small algal samples that were insufficient to make a 4.5 mg sample.

Macroinvertebrate consumers were handpicked off of various substrates, including *P. ceratophyllum, Cladophora,* wood, leaves, and rocks in the river channel. Consumers were transported to the lab alive and placed in aquaria in lab minimally for 24 hrs to allow their gut to clear. This allows any undigested food to pass through their gut and prevent the stable isotope results from including components of their diets that are not assimilated into body tissue. Consumers were euthanized after this time in hot water and separated by taxa.

All samples were placed in ceramic crucibles and into a drying oven at 70^0 C for at least 48 hours. Dried samples were pulverized to a fine powder using a Wig-L-Bug®., measured out into two separate sample sizes for food resources (4.5 mg) and consumers (1.5 mg), and placed into 5x9 mm tin capsules. Carbon and nitrogen stable isotopic ratios were performed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility, University of Davis, California, USA. Stable isotope ratios were calculated as $\delta^{13}C$ or $\delta^{15}N$ (per mil) = $([R_{sample}/R_{standard}]-1) * 1000$, where R is the ^{13}C:^{12}C ($=\delta^{13}C$) or ^{15}N:^{14}N ($=\delta^{15}N$) ratio. Pee Dee Belemnite and atmospheric nitrogen (AIR) were used as standards for carbon and nitrogen analysis, respectively. Machine error for $\delta^{13}C$ was 0.2‰; machine error for $\delta^{15}N$ was 0.3‰.

Statistical analyses

Percent cover of *Cladophora* and *P. ceratophyllum* was compared between the four study reaches using a non-parametric ANOVA. This statistical technique used a residual randomization procedure (RRP) with 9999 randomized permutations to produce a null distribution, which is then compared with the observed result. The p-value is determined by computing the rank percentile of the observed result within the distribution. The p-value was considered significant if $\alpha \leq 0.05$.

Niche breadth was computed by calculating the $\delta^{13}C$ range of primary consumers collected at each reach (Layman et al. 2007). The $\delta^{13}C$ range is expected to be wider in environments with several food resources with unique $\delta^{13}C$ signatures, which allows primary consumers to have diverse trophic niches (Layman et al. 2007). Stable isotope data for primary consumers were compared to data from potential food resources collected at the same study reach using IsoSource using procedures outlined in Phillips & Gregg (2003). Trophic enrichment factors of 0.4 for $\delta^{13}C$ and 3.4 for $\delta^{15}N$ were added to all food resources prior to analyses to account for fractionation rates during digestion and assimilation processes (Phillips & Gregg 2003). Increment and tolerance levels were initially set at 1% and 0.2% for each analysis, respectively. When consumer isotopic values fell outside of mixing polygons, tolerance levels were increased by increments of 0.1% until either IsoSource produced feasible results or the tolerance level reached 0.7%.

Potential food resources included in analyses were leaves, *Cladophora*, *P. ceratophyllum* epilithic biofilm, wood biofilm, surface BOM, fine BOM, coarse BOM, and TOM. Data from algal and detrital fractions of wood biofilm were combined since isotope values were nearly identical. All deep BOM fractions except ultrafine (<1µm)

were combined since isotopic values were nearly identical. Data from all three species of tree leaves were also combined.

Due to the large number of potential food resources, it was impractical to use every food resource for each IsoSource run. A smaller suite of potential food resources was chosen based off of the feeding habits and mouthpart morphologies of individual consumer taxa. Potential food resources for *Cheumatopsyche, H. simulans,* and *Isonychia* included *Cladophora, P. ceratophyllum,* tree leaves, TOM, wood biofilm, epilithic biofilm, fine BOM, and coarse BOM. Potential food resources for *C. fluminea* included *Cladophora,* wood biofilm, epilithic biofilm, TOM, and fine BOM. Food resources for all shredder taxa (*P. dorsata, Pycnopsyche,* and *Lepidostoma*) included *Cladophora, P. ceratophyllum*, tree leaves, wood biofilm, epilithic biofilm, fine BOM, and coarse BOM. Most of the scraper taxa (*L. praerosa, Maccaffertium, P. herricki, Stenacron,* and pleurocerid snails) had *Cladophora*, *P. ceratophyllum*, wood biofilm, epilithic biofilm, fine BOM, and coarse BOM as potential food resources. Potential food resources for the scraper taxa *Optioservus* also included leaves. IsoSource results were displayed as ranges of resource contributions (1-99th %tiles of all feasible results).

Results

Increasing karstification and longitudinal gradients in $\delta^{13}C$ values

Mean primary consumer and *P. ceratophyllum* $\delta^{13}C$ values became increasingly ^{13}C-depleted in downstream reaches (Tables 4–5). While changes in consumer and in-stream producer $\delta^{13}C$ values cannot be clearly traced to DIC gradients in the Green River,

Finlay (2001) suggested that longitudinal changes in DIC abundances may often cause gradients in algal $\delta^{13}C$ values.

Percent cover measurements

Cladophora cover varied significantly between reaches during autumn 2013, but cover was low in all reaches (F value=6.48, r^2=0.357, p <0.01). *Cladophora* cover in both upstream reaches was nearly zero and was significantly less than in the furthest downstream reach, but results were only weakly significant (Figure 5). The first downstream reach had intermediate amounts of *Cladophora* cover and was not significantly different than any of the other three reaches. Frequent rains during summer and autumn 2013, leading to periodic flow events that were markedly higher than the previous year periodically scoured the riverbed, dislodging most *Cladophora* from all reaches (Figure 2). Small amounts of *Cladophora* were present near river banks and in areas protected from scouring, but *Cladophora* was visually scarce and rarely grew <1m long in all reaches.

Podostemum ceratophyllum cover also varied significantly between reaches during this study period (F-value=34.57, r^2=0.748, p<0.001). The furthest upstream reach was significantly higher than all other reaches, including the second upstream reach located only 2 km downstream (Figure 4). Cover in the second upstream reach was significantly higher than the two downstream reaches, and the downstream two reaches were not significantly different from each other.

Longitudinal gradients in niche breadth:

While this study predicted that increasing in *Cladophora* abundance downstream may be correlated with decreasing consumer niche breadths, results from this study did not support this. Niche breadth was similar in three out of the four reaches (Table 6). The second upstream reach had markedly increased niche breadth because one consumer, *P. herricki*, was markedly more ^{13}C-enriched than other consumers for unknown reasons (Tables 5–6). Had this taxon been excluded from the analysis, niche breadth in this reach would have been comparable to the other three.

Longitudinal gradients in Cladophora assimilation

Isotopic longitudinal trends:

Longitudinal gradients in consumer isotopes did not provide clear evidence of a longitudinal shift in diet. Mean consumer $\delta^{13}C$ values become increasingly ^{13}C-depleted downstream (Table 6). These longitudinal gradients, however, coincided with similar longitudinal gradients in at least one potential food resource. *Podostemum ceratophyllum* also became increasingly ^{13}C-depleted in downstream reaches (Figure 46). The $\delta^{13}C$ values of other potential food resources, including *Cladophora*, did not display any longitudinal pattern (Figure 46).

Longitudinal trends in IsoSource results:

The mixing model analyses from IsoSource did not indicate that consumers assimilated less *Cladophora* in upstream reaches than downstream reaches. Feasible solutions for *Cladophora* generally ranged between a minimum near 0% to a maximum of less than 50% in most taxa (Figures 48–55, 57–61). Two exceptions were *C. fluminea*

and *P. dorsata*, which had higher *Cladophora* contributions in some reaches for unknown reasons (Figures 49, 56). No taxa had visibly increased *Cladophora* contributions in downstream reaches (Figures 48–61).

Consumer diets: autochthonous vs. allochthonous resources

Longitudinal gradients in consumer and resource isotopes

Longitudinal gradients in consumer and resource $\delta^{13}C$ values suggested that consumers assimilated at least one autochthonous resource. Mean consumer $\delta^{13}C$ values become increasingly ^{13}C-depleted downstream, which coincided with similar patterns in *P. ceratophyllum* (Table 6, Figure 46). The $\delta^{13}C$ values of other potential food resources, including *Cladophora*, did not display any longitudinal pattern (Figure 46).

Longitudinal patterns in consumer isotopes, however, did not provide any information on whether consumers assimilated allochthonous food resources. Longitudinal gradients in consumer isotopes did not match any existing gradients in isotopic values of allochthonous resources. While tree leaves became slightly ^{15}N-enriched in downstream reaches, primary consumer $\delta^{15}N$ values displayed no longitudinal gradient (Figure 46). Other allochthonous resources displayed no clear longitudinal gradients.

IsoSource results

The mixing model analyses from IsoSource did not identify consistent prominent food resources for communities of consumer taxa in the upper Green River. IsoSource results typically resulted in similar ranges for all food resources. Ranges of feasible

contributions for most food resources usually ranged between 0% to a maximum of ca. 20–50%. While some IsoSource analyses indicated the presence of a prominent resource, the food resources varied by individual taxa.

IsoSource results: Reach A

IsoSource produced feasible results for seven out of eight taxa in the furthest upstream reach (Figures 49, 51, 53–55, 57, 60). Data for *H. simulans* did not produce any feasible results, although the consumer isotope value was within the mixing polygon (Figures 50, 64). Only four of the seven successful analyses indicated that the consumer taxa had a clear prominent food resource. Analysis of the consumer *P. herricki* only returned one feasible solution, which suggested that this consumer assimilated mostly coarse BOM and rock biofilm (Figure 57). The prominent food resource for *Maccaffertium* appeared to be coarse BOM (Figure 54). BOM visually appeared woody and derived mainly from terrestrial resources. Results for *C. fluminea* suggested that this consumer largely assimilated *Cladophora* (Figure 49). *Isonychia*, *Optioservus*, and *L. praerosa* results did not display any clear prominent food resource, though the maximum contributions for rock and wood biofilms were noticeably higher than other food resources in the analysis of *L. praerosa* (Figure 51, 53, 55).

IsoSource results: Reach B

Ten out of eleven consumer taxa in the second upstream reach produced feasible solutions in IsoSource. Data from *P. herricki* did not produce feasible results, although the stable isotopes were within the mixing polygon (Figure 71). This consumer was more

^{13}C-enriched than all the food resources except rock biofilm. Five out of the ten taxa produced IsoSource results that showed no prominent food resource (Figure 48, 55–56, 58–59). Feasible contributions for each food resource generally ranged between a minimum of 0% and a maximum ca. 20–50%. Results for *L. praerosa* were an exception. Although minimum and median contributions for each food resource were relatively low, the maximum contributions from wood biofilm and fine BOM were higher (Figure 53). Consumers that displayed prominent food resources appeared to vary in diet according to individual taxa. Results for *C. fluminea* suggested that it largely assimilated *Cladophora* and TOM (Figure 49). Resource contributions for *P. ceratophyllum* were noticeably higher than other food resources in the analysis of *H. simulans* (Figure 50). Leaves appeared to be a prominent food resource for *Isonychia* (Figure 51). The prominent food resource for *Maccaffertium* appeared to be coarse BOM (27–69%) (Figure 54).

IsoSource results: Reach C

Ten out of the eleven consumer taxa from the first downstream reach produced IsoSource results. *Psephenus herricki* did not produce feasible IsoSource results because it was more ^{15}N-depleted than all its food resources (Figure 71). Five of the ten remaining consumer taxa lacked a prominent food resource (Figures 50, 52–53, 56, 58). The remaining four taxa had prominent food resources that varied according to individual taxa. *Isonychia* results displayed slightly higher resource contributions from leaves (Figure 51). *Maccaffertium* results displayed higher resource contributions from coarse BOM (Figure 54). Results indicated that the prominent food resources for Pleuroceridae-wrinkled were wood biofilm and fine BOM (Figure 60), while Pleuroceridae- smooth

appeared to assimilate largely fine BOM (Figure 61). Results at this reach indicated that TOM was a prominent food resource for *C. fluminea* (Figure 49).

IsoSource results: Reach D

Nine out of eleven taxa in the second downstream reach produced feasible IsoSource results. *Leptoxis praerosa* did not produce any feasible solutions in IsoSource even though the consumer was inside the mixing polygon (Figure 67). *Lepidostoma* did not produce feasible IsoSource, and it was outside the mixing polygon because it was more ^{15}N-depleted than most food resources when trophic enrichment factors were added (Figure 66). Four out of the nine consumer taxa with feasible solutions showed similar source contributions for all food resources (Figures 50, 57, 60–61). Four taxa had results that indicated that leaves were a prominent food resource (Figures 51, 55–56, 58). In addition to leaves, *Cladophora* is also a prominent food resource for *P. dorsata* (Figure 56). Results for *C. fluminea* indicated that this consumer assimilated largely *Cladophora* and fine BOM (Figure 49).

Mixing polygons

When consumer stable isotopes were plotted in mixing polygons, most consumers were near the center of the mixing polygon. Consumer isotopic values in most reaches fell within the ranges of resource stable isotopes. *Cladophora* and *P. ceratophyllum* were both more ^{13}C-depleted than other food resources. Tree leaves had intermediate δ^{13}C values between *Cladophora* and *P. ceratophyllum* and the other food resources. Wood and rock biofilm, both fractions of BOM, and TOM all were more ^{13}C-enriched and had

similar $\delta^{13}C$ values. Unlike 2012, where consumers were more ^{13}C-depleted than the mean resource $\delta^{13}C$ value, primary consumer $\delta^{13}C$ values were similar to that of the mean food resource.

Consumer and resource $\delta^{13}C$ values

Food resources collected at the furthest upstream reach had $\delta^{13}C$ values that ranged between −36.64‰±0.01 (*P. ceratophyllum*) and −20.52‰±0.21 (rock biofilm) (Table 4). Primary consumers at this reach ranged between −31.32‰±0.18 (*C. fluminea*) and −24.85‰±0.06 (Pleroceridae- wrinkled) (Table 5). Food resources in the second upstream reach had $\delta^{13}C$ values that ranged from −43.34‰±0.01 (*Cladophora*) to −11.93‰±0.14 (rock biofilm) (Table 4). Primary consumer $\delta^{13}C$ values ranged from −32.94‰±0.05 (*P. dorsata*) to −21.81‰±0.04 (*P. herricki*) (Table 5). Food resources at the first downstream reach had $\delta^{13}C$ values between −44.00‰±0.01 (*Cladophora*) and −27.54‰±0.09 (wood biofilm) (Table 4). Benthic organic matter was not sampled at this reach due to high water levels at the time of sampling. The fine BOM from the furthest downstream reach was more ^{13}C-enriched than all the food resources at the first downstream reach. It is likely that this food resource would also have been the most ^{13}C-enriched food resource at the first downstream reach. Primary consumer $\delta^{13}C$ values in the first downstream reach ranged between −33.59‰±0.07 (*P. dorsata*) and −26.89‰±0.03 (*Pleuroceridae*- smooth shell) (Table 5). Food resource $\delta^{13}C$ values at the furthest downstream reach ranged between −38.31‰±0.05 (*P. ceratophyllum*) and −25.14‰±0.01 (rock biofilm) at the furthest downstream reach (Table 4). The $\delta^{13}C$

values of consumers ranged between −33.31‰±0.01 (*P. dorsata*) and −27.60‰±0.04 (Pleuroceridae- smooth shell) (Table 5).

Consumer and resource $\delta^{15}N$ values

Food resources collected at the furthest upstream reach had $\delta^{15}N$ values that ranged between 0.83‰±0.28 (tree leaves) and 8.24‰±0.02 (*Cladophora*) (Table 4). Consumers at this reach had $\delta^{15}N$ values that ranged from 7.96‰±0.04 (*P. herricki*) and 10.65‰±0.16 (*C. fluminea*) (Table 5). Resource $\delta^{15}N$ at the second upstream reach ranged from 0.83‰±0.28 (tree leaves) and 8.73‰±0.01 (*P. ceratophyllum*). Consumer $\delta^{15}N$ values ranged between 7.96‰±0.09 (*P. herricki*) and 10.81‰±0.02 (*H. simulans*). Resources at the first upstream reach ranged between 2.31‰±0.30 (tree leaves) and 8.40‰±0.04 (*P. ceratophyllum*). Consumers at this reach ranged between 6.95‰ (*S. crenata* group) and 9.86‰±0.04 (Pleuroceridae- wrinkled). Resources in the furthest downstream reach ranged between 2.31‰±0.30 (tree leaves) and 10.28‰±0.02 (*P. ceratophyllum*). Consumers at this reach had $\delta^{15}N$ values that ranged between 5.91‰±0.06 (*Lepidostoma*) and 10.76‰±0.03 (*H. simulans*).

IsoSource results: Functional feeding groups

Overall, IsoSource analyses indicated that the three functional feeding groups represented in this sample set did not assimilate differing amounts of *Cladophora*. Five out of the eleven analyses for filter feeders had results that suggested that the minimum contribution for *Cladophora* was >0% (Figures 48-51). Four out of these five analyses came from *C. fluminea* (Figure 49). These four analyses suggested that this consumer

assimilated *Cladophora* and TOM or fine BOM. Most analyses of other taxa displayed no prominent food resource (Figures 48, 50-51). Two analyses from *Isonychia* showed slightly higher contributions from leaves (Figure 51), and one analysis from *H. simulans* had higher source contributions for *P. ceratophyllum* (Figure 50).

Five of the seven IsoSource analyses for shredder taxa had minimum source contributions for *Cladophora* that were >0% (Figures 52, 56, 58).Maximum *Cladophora* contribution varied between 28-55% and median values varied widely between 4-55%. Scrapers had fewer analyses with large *Cladophora* contributions. Only two of the seventeen analyses had minimum *Cladophora* contributions >0% (Figure 54). These two analyses had relatively low medians for *Cladophora* contribution (15%). Scraper taxa generally had no prominent food resources or tended to have higher source contributions from BOM, biofilm, or leaves (Figures 53-55, 57, 59, 60-61).

Discussion

Increasing karstification and longitudinal gradients in $\delta^{13}C$ values

Observed trends in primary consumer and *P. ceratophyllum* $\delta^{13}C$ values were surprising. Algal $\delta^{13}C$ values can vary greatly, and longitudinal gradients in algal $\delta^{13}C$ values are common since conditions vary between reaches (France 1995; Finlay et al. 1999; Finlay 2001). One meta-analysis by Finlay (2001) concluded that algae became increasingly ^{13}C-enriched downstream. The $\delta^{13}C$ values of *P. ceratophyllum* and primary producers in this study, however, showed the opposite trend.

Primary producer $\delta^{13}C$ values can be influenced by several factors, including growth rate, species, water velocity, and supply of DIC (Finlay et al. 1999; Finlay 2001; Finlay 2004; Singer et al. 2005). DIC can influence primary producer $\delta^{13}C$ values

primarily in two ways: by the $\delta^{13}C$ values of DIC itself and the abundance of DIC that is available to primary producers. The $\delta^{13}C$ values of DIC in the Green River are unlikely to contribute to longitudinal patterns in producer and consumer $\delta^{13}C$ values. One study found that $\delta^{13}C$ values of DIC were similar in all reaches and averaged between −12‰ to −14‰, depending on the season (McClanahan 2014). This was slightly surprising, given that downstream reaches had more homogenous carbonate lithology than upstream reaches (Osterhoudt 2014). Groundwater and surface streams in limestone karst regions are expected to have DIC with relatively ^{13}C-enriched values due to DIC from carbonate rock, since limestone usually has a $\delta^{13}C$ value around 0‰ (Clark & Fritz 1995; Dubois et al. 2010). Despite more homogenous carbonate lithology in downstream reaches, the largest source contributor of DIC was soil respiration in all reaches (McClanahan 2014).

While trends in primary producer and consumer $\delta^{13}C$ values in the Green River cannot be definitively traced to any one cause, longitudinal gradients in algal $\delta^{13}C$ values sometimes occur as a result of longitudinal gradients in DIC availability. The abundance of DIC influences primary producer $\delta^{13}C$ values by changing fractionation rates during photosynthesis. Differential fractionation occurs because ^{12}C is preferentially used during photosynthesis (Fry 2007). Carbon limitation will decrease fractionation rates and result in more ^{13}C-enriched values. Conversely, increasing availability of DIC to autotrophs during photosynthesis increases fractionation since more ^{12}C is available for photosynthesis (Finlay 2001). Increased concentration of DIC or increased water velocity is correlated with more ^{13}C–depleted values in algae (Hemminga & Mateo 1996; Burkhardt et al. 1999; Finlay et al. 1999; Riebesell et al. 2000; Finlay 2001). Increased

water velocity, however, only has strong effects on algal $\delta^{13}C$ values when algae are carbon limited (Finlay et al. 1999).

A study by Finlay (2001), which included data from 70 study reaches and 26 published studies, revealed that algal $\delta^{13}C$ values increased with watershed area (Finlay 2001). Watershed area was used as a proxy for DIC abundance. The concentration of CO_2 is typically lower in downstream reaches relative to upstream (Lorah & Herman 1988; Dawson et al. 1995; Jones & Mulholland 1998b; Finlay et al. 1999). This was the suggested cause of algal and consumer longitudinal trends within these studies.

While most streams have decreasing DIC availability in downstream reaches, some factors may either mitigate or even reverse this trend. Abundances of DIC may actually increase downstream in the Green River, which would likely lead to observed trends in producer and consumer $\delta^{13}C$ values. One potential factor that may have decreased DIC concentrations upstream was the Green River Dam. All study reaches were between 43 and 155 km downstream of the Green River Dam. Lakes and reservoirs consume DIC both through processes within the lake and by degassing due to long residence times, decreasing the amount of DIC downstream (Yang et al. 1996; Barth & Veizer 1999; Aucour et al. 1999; Kling et al. 2000). No data on DIC availability was collected upstream of the Green River Dam, however, so effects of this impoundment on water composition are uncertain.

The abundances of DIC may also increase downstream if downstream reaches receive large inputs of DIC-rich water or where rivers transition from a siliciclastic to carbonate lithologies. In one riverine system, DIC abundances increased downstream due to large inputs of water from poorly-degassed tributaries (Flintrop et al. 1996). Similarly,

a number of large karst springs add large inputs of water into downstream reaches of the Green River, which is most likely poorly-degassed and DIC-rich (Blair et al. 2012). Spring inputs often have large amounts of DIC (Rounick & James 1984). Dissolved inorganic carbon may also become more available downstream if the underlying lithologies transition from siliciclastic to carbonate bedrocks. While increasing discharge is typically related to decreases in DIC, concentrations of DIC are expected to increase with increasing karstification (Telmer & Veizer 1999; Schulte et al. 2011; Florea 2013). The upper Green River transitions from a more heterogeneous lithology upstream watershed to an almost purely carbonate-dominated lithology in downstream reaches (Figure 1). During one study during winter 2013, DIC concentrations in one of the upstream reaches ranged from 30-95 (mg/L), and between 60-140 (mg/L) in a downstream reach (Osterhoudt 2014). While these measurements were taken during a short term study in winter, these longitudinal trends are consistent with findings from other studies and may persist in other seasons. If these longitudinal gradients in DIC availability are consistent, then primary producer and consumer $\delta^{13}C$ values are likely influenced by these patterns.

While no definitive explanation can be given for longitudinal gradients in *P. ceratophyllum* and consumer $\delta^{13}C$ values, primary producers may have become more ^{13}C-depleted downstream in response to increasing concentrations of DIC. While DIC concentrations decrease downstream in most streams, influences such as changes in lithology and large karst spring inputs may potential reverse typical longitudinal gradients. Further investigations into the role of karst lithology on DIC availability and

algal carbon isotopic values may reveal exceptions to the trends published in Finlay (2001).

Cladophora $\delta^{13}C$ values did not have any clear longitudinal gradient. This algae probably only reflects extremely recent fluctuations in DIC abundances because of a quick growth rate and, therefore, quick turnover rate in $\delta^{13}C$ values (Dodds & Gudder 1992; Hadwen et al. 2010). Under more stable hydrological conditions, algae $\delta^{13}C$ values may have reflected more stable long term averages in $\delta^{13}C$ values.

Percent cover

Karst geology is often associated with higher nutrient inputs and high algal accrual, including *Cladophora* (Leland & Porter 2000; Notestein et al. 2003; Penick 2012). *Cladophora*, a type of filamentous green algae, is common in high nutrient waters and can be an important food resource for macroinvertebrate consumers (Rhame & Stewart 1976; Dodds 1991). Several high water events during the study period, however, caused *Cladophora* cover to be sporadic and an overall less abundant food resource (Figure 2). Results from this study did not support the hypotheses that consumers had lower niche breadths downstream and assimilated more *Cladophora.*

Cladophora cover was surprisingly low in all reaches compared with other studies in previous years (Penick et al. 2012; Yates 2012; Tinsley 2012). Mean cover was 0% in both upstream reaches and 0.6 and 1.4% in the downstream reaches (Figure 5). Discharge rates were much more variable in the Green River during summer 2013 than during summer 2012 (Figure 2). *Cladophora* is susceptible to scouring, and frequent high water events immediately prior to and during the study periodically removed *Cladophora*

growth downstream (Power 1990; Ensmingner et al. 2000; Power et al. 2009) (Figure 5). Despite higher *Cladophora* availability in downstream reaches, this food resource was not markedly abundant at any of the four reaches.

Podostemum ceratophyllum standing stocks were relatively abundant at all reaches but significantly decreased downstream (Figure 4). High water events preceding the 2013 study period may have led to bedload movement of sand and gravel, which appeared to accumulate in the downstream reaches and the subsequent burial of *P. ceratophyllum* beds. The relative loss of cobble substrates in the study riffles are being attributed to burial by smaller substrates and not downstream movement. Hence, cobbles and the *P. ceratophyllum* beds they support via habitat were lost to burial.

Longitudinal gradients in niche breadth

Similarly, niche breadth did not display a longitudinal gradient. Niche breadth was similar in all reaches, except for the second upstream reach (Table 6). The second upstream reach had a much wider niche breadth because one consumer, *P. herricki,* was much more ^{13}C-enriched than the other consumers (Table 5). If this consumer was excluded from analyses, niche breadth in all reaches. Niche breadth was expected to decrease in downstream reaches during *Cladophora* blooms due to longitudinal gradients in *Cladophora* abundance. Consumers that are generalists tend to feed on foods according to availability (Feinsinger et al. 1981). Algal blooms may cause a few species of producers to be extremely abundant relative to other food resources, which may then cause consumers to rely increasingly more on a few dominant species. *Cladophora* cover remained low during autumn 2013, however (Figure 5). With only slight differences in

Cladophora cover between upstream and downstream reaches, these results are not surprising.

Longitudinal gradients in Cladophora assimilation

Longitudinal patterns in consumer stable isotopes did not provide evidence of a change in diet. Both reach-wide mean consumer $\delta^{13}C$ and $\delta^{15}N$ values displayed longitudinal gradients (Figure 46). Mean consumer $\delta^{15}N$ values were slightly lower downstream, but the range was <1‰ between the furthest upstream and furthest downstream reaches (Table 5). Mean consumer $\delta^{13}C$ values were increasingly ^{13}C-depleted in downstream reaches (Figure 47). Shifts in stable isotopes, particularly in carbon isotopes, are often associated with diet changes (Phillips & Eldridge 2006; Del Rio et al. 2009). These longitudinal shifts in $\delta^{13}C$ values of consumers, however, could be accounted for by similar patterns in at least one autochthonous food resource (Figure 46). Consumer isotopic values track that of food resources (Finlay 2001), and longitudinal gradients in this study were likely a result of this. Analyses using IsoSource, furthermore, suggested that *Cladophora* assimilation was not markedly different between reaches (Figures 48-61).

These findings contrast with predictions that consumers would assimilate greater amounts of *Cladophora* in downstream reaches. Stable isotope analyses during previous blooms indicated that consumers relied greatly on this food resource in the furthest downstream reach (Penick et al. 2012; Tinsley 2012; Yates 2012). During this study period, however, frequent spates periodically washed new *Cladophora* growth (Figure 2).

Cladophora percent cover never exceeded 5% during line transect measurements (Figure 5). Consumer diets at all reaches seem to reflect the low availability of this resource.

Consumer diets: autochthonous vs. allochthonous resources

Longitudinal gradients in consumer isotopes

Longitudinal gradients in consumer $\delta^{13}C$ values provided partial evidence for assimilation of at least one autochthonous resource. Algal food resources have a much wider range in $\delta^{13}C$ values than terrestrial resources, and longitudinal gradients in isotopes are common in streams (France 1995; Finlay et al. 1999; Finlay 2001). Consumers that assimilate algae share similar isotopic patterns (McCutcheon & Lewis 2002). Resource $\delta^{13}C$ values only partially support this explanation. *Podostemum ceratophyllum* $\delta^{13}C$ values become more ^{13}C-depleted in downstream reaches (Table 4). All other algal food resources, including *Cladophora*, showed no clear longitudinal pattern.

Algal $\delta^{13}C$ values may not accurately reflect longer term longitudinal gradients because of quick turnover rates, especially when growth is rapid. Algal $\delta^{13}C$ values are affected by a number of environmental factors, including water velocity (Finlay & Kendall 2007). Consumer tissues reflect the $\delta^{13}C$ values of food resources assimilated over a relatively long period of time, and have slower shifts in $\delta^{13}C$ values than basal food resources (Fry & Arnold 1982; Hesslein et al. 1993; Wainright et al. 1993). Changes in algal $\delta^{13}C$ values occur especially quickly in eutrophic waters or where discharge is varied, and consumer $\delta^{13}C$ values may not respond quickly enough to accurately reflect the values of current food resources in these conditions (Singer et al. 2005). Periodic high

water events frequently removed most existing *Cladophora* during this study. Fairly recent *Cladophora* growth that was collected for stable isotopic analyses more likely reflected immediate and extremely short term DIC availability rather than the longer-term mean $\delta^{13}C$ values found in primary consumer tissues. *Podostemum ceratophyllum*, in contrast, has a slower metabolism and a stable holdfast root system (Hill & Webster 1982b; 1984). This macrophyte was present throughout the growing season despite high water levels. The $\delta^{13}C$ values of *P. ceratophyllum* were most likely influenced by past DIC availability as well as present growing conditions.

IsoSource results

Mixing models suggest that consumers assimilated multiple food resources during this study period (Figures 48-61). Isotope studies may lack clear results either where food resource isotopes overlap or where consumers are at the center of the mixing polygon (Fry 2007; Winemiller et al. 2007). Wood biofilm, both fine and coarse BOM, and TOM had highly similar $\delta^{13}C$ values. Epilithic biofilm also had similar $\delta^{13}C$ values to these food resources in the two downstream reaches (Table 4). This cluster of food resources was more ^{13}C-enriched than other food resources. Most consumer isotope ratios fell within the center of the mixing polygon, creating IsoSource solutions that had wide feasible ranges and fairly low medians for all food resources (Figures 62-77). Mixing polygons indicated that consumers most likely assimilate a combination of ^{13}C-depleted resources, particularly *Cladophora* or *P. ceratophyllum*, at least one ^{13}C-enriched resource, and terrestrial tree leaves, the latter of which are more ^{15}N-depleted than other

food resources. While a few consumer samples did appear to have a single prominent food resource, this resource appeared to differ greatly between both taxa and reach.

These findings contrast with previous studies conducted during summer and autumn in these reaches. *Cladophora* is often abundant during this part of the year in downstream reaches, and stable isotope analyses during previous blooms indicated that consumers relied greatly on this food resource (Penick et al. 2012; Tinsley 2012; Yates 2012). During this study period, however, frequent high water events periodically washed new *Cladophora* growth downstream and consumer diets seem to reflect the low availability of this resource. Many primary consumers are able to use and assimilate a number of food resources (Sterner & Hessen 1994; McNeely et al. 2007). Scouring lessened the abundance of food resources normally prolific during this season, and consumer taxa likely adjusted their diets accordingly by shifting to other abundant resources.

Although source contributions for *Cladophora* and *P. ceratophyllum* are less prominent food resources than in previous years, longitudinal gradients and mixing polygons suggest that they are still important basal resources. *Cladophora* is sometimes directly consumed by macroinvertebrates (Marcus et al. 1978; Gray & Ward 1979; Dodds 1991; McShaffrey & McCafferty 1991; Dodds & Gudder 1992). In contrast, *P. ceratophyllum* is not usually directly grazed (Hutchens et al. 2004). Studies indicate that both *P. ceratophyllum* and *Cladophora* may also indirectly supply food for invertebrates by acting as a substrate for epiphytes (Dodds 1991; Hutchens et al. 2004; Roll et al. 2005; Furey et al. 2012). Epiphytes were found on both *P. ceratophyllum* and *Cladophora* during the study period and were not removed from samples.

Consumer diets: summary

Overall, consumers appear to partially rely on algal food resources. Midreaches generally have high rates of productivity and high availability of algal resources due to increased light availability (Vannote et al. 1980, Kohler et al. 2012). Since algal resources are abundant and generally more labile than terrestrial leaf litter, consumers at these reaches are expected to assimilate high proportions of in-stream producers (Vannote et al. 1980; Lewis et al. 2001; Bunn et al. 2003). Frequent peak flow events during the growing season, however, appear to have slightly modified consumer diets. These spates washed *Cladophora* growth downstream, removing the majority of an abundant food resource (Figure 5). Moderate spates can actually increase growth of other types of algae, such as diatoms, because of increased nutrient input (Stevenson 1990). However, the effects of frequent, extreme spates are less clear and could possibly scour large amounts of algae. Although cover was severely reduced, consumers appear to assimilate at least one major in-stream primary producer.

Summary

Consumer and *P. ceratophyllum* $\delta^{13}C$ values became more ^{13}C-depleted in downstream reaches. While the cause of this pattern cannot be definitively identified, longitudinal gradients in DIC availability have been linked with longitudinal trends in $\delta^{13}C$ values of algae in other streams (Finlay 2001). Observed trends in this study, however, were opposite to typical longitudinal trends in algal $\delta^{13}C$ values, suggesting that DIC availability may have increased in downstream reaches. Increasing karstification and

large inputs from karst springs in downstream reaches may have reversed typical longitudinal trends in DIC availability, though future studies will be needed to confirm this.

Frequent peak flow events during the study period periodically scoured *Cladophora,* limiting the abundance of this food resource. Isotopic analyses suggested that primary consumers had similar diets and niche breadths in all reaches. Mixing model results were unclear and displayed small to moderate assimilation of each food resource. Consumers appeared to assimilate a mix of autochthonous and allochthonous resources. What remains unclear, however, is which in-stream primary producers were assimilated by consumers. Consumers may assimilate *P. ceratophyllum, Cladophora,* associated epiphytes, or a combination of these resources.

Although consumers are expected to rely largely on autochthonous resources in midreaches, mixing model analyses also suggested that allochthonous resources such as tree leaves may be important resources as well. These results suggest that consumers in midreaches may still supplement their diets with allochthonous resources, especially during periods with lower algal growth.

CHAPTER 4: CONCLUSIONS

Longitudinal trends: stable isotopic values

Carbon isotopic values are commonly used as tracers for consumer diets, but increasing support suggests that changes within algal and consumer $\delta^{13}C$ values may also provide information on DIC sources and availability in streams (Finlay 2001; Fry 2007). Finlay (2001) found that algae and primary consumer $\delta^{13}C$ values typically became increasingly ^{13}C-enriched in downstream reaches. These were correlated with longitudinal gradients in DIC abundance rather than changes within DIC $\delta^{13}C$ values. Larger watersheds typically have lower concentrations of DIC (Lorah & Herman 1988; Dawson et al. 1995; Jones & Mulholland 1998; Finlay et al. 1999), and algae that are carbon-limited subsequently become increasingly ^{13}C-enriched. Conversely, algae in headwater streams are often ^{13}C-depleted because of high DIC availability. Photosynthetic rates are often limited by shading and spring inputs can bring water that is supersaturated with CO_2 (Vannote et al. 1980; Rounick & James 1984; Lamberti & Steinman 1997).

Primary consumers and at least one primary producer in this study had $\delta^{13}C$ values that displayed longitudinal gradients during both study periods. Instead of becoming more ^{13}C-enriched, however, carbon isotopic values actually became more ^{13}C-depleted in downstream reaches. While this study cannot definitively determine a single cause for this pattern, these trends may result from an increase in DIC abundance downstream. Such reversals in DIC gradients can occur if upstream impoundments or lakes decrease the supply of DIC, or the underlying landscape shifts from siliciclastic to carbonate lithologies (Yang et al. 1996; Barth & Veizer 1999; Telmer & Veizer 1999;

Aucour et al. 1999; Kling et al. 2000; Schulte et al. 2011). All study reaches were 43 to 155km downstream of the Green River dam, but no data on DIC upstream of the dam are currently available for analysis. Because of relatively-fast weathering rates, carbonate rocks in particular can have a profound influence on inorganic carbon concentrations in a watershed (Roy et al. 1999). The upper Green River transitions between a siliciclastic to highly-carbonate dominated landscape, which results in increased carbonate dissolution downstream (Schulte et al. 2011; Osterhoudt 2014). DIC concentrations in the upper Green River actually increased longitudinally during a brief study in winter 2013 (Osterhoudt 2014), and while no other data exists on longer-term trends, this pattern may remain consistent during other seasons as well. The Green River also has several large karst springs in the downstream reaches (Blair et al. 2012). Spring inputs are often supersaturated with DIC and likely provide large inputs of poorly-degassed water into downstream reaches (Rounick & James 1984). The $\delta^{13}C$ values of DIC, in contrast, were very similar between upstream and downstream reaches in a concurrent study, and therefore would not explain gradients in algal $\delta^{13}C$ values (McClanahan 2014).

Increasing availability of DIC would allow primary producers to select for ^{12}C during photosynthesis, thereby increasing fractionation rates (Finlay 2001). *Cladophora* and *P. ceratophyllum* were more $\delta^{13}C$-depleted in downstream reaches during summer 2012. *Podostemum ceratophyllum* also became more $\delta^{13}C$-depleted in downstream reaches during autumn 2013, but *Cladophora* displayed no longitudinal gradient that year. Mean *Cladophora* stable isotopes over a larger period of time may have reflected similar longitudinal patterns to those during summer 2012. Algal stable isotopes can change rapidly in response to environmental conditions, especially under variable

hydrological conditions in eutrophic systems (Singer et al. 2005). Due to high growth rates of *Cladophora* and scouring, which periodically removed most *Cladophora* biomass, stable isotopic values of this algae likely reflected immediate DIC concentrations rather than a longstanding average (Singer et al. 2005; Hadwen et al. 2010).

Similarly, primary consumers became increasingly ^{13}C-depleted downstream during both years. Primary consumers that rely on algal resources have similar longitudinal $\delta^{13}C$ trends as algae (Finlay 2001). Consumer tissues, however, reflect the $\delta^{13}C$ values of food resources assimilated over a relatively long period of time, and have slower shifts in $\delta^{13}C$ values than basal food resources (Fry & Arnold 1982; Hesslein et al. 1993; Wainright et al. 1993). Consumer $\delta^{13}C$ values additionally provide evidence that longitudinal gradients remained over a period of time and occurred during two different years.

While underlying lithology and resulting increases in DIC are a likely cause for observed gradients in algal $\delta^{13}C$ values, further analyses are needed to clearly find a link between these trends. Although current evidence suggests that DIC may increase downstream, more data is needed to confirm that this pattern is stable over multiple seasons. Still more studies are needed to differentiate whether gradients in DIC are a result of decreases in DIC upstream due to the Green River dam, or whether these gradients result from DIC-rich inputs downstream due to increasing karstification.

Longitudinal trends: niche breadth

Niche breadth differed between the two study years, but neither year displayed a strong longitudinal gradient (Tables 3, 6). High abundance of *Cladophora* downstream was predicted to correlate with decreased niche breadths of primary consumers. Niche breadth was slightly narrower in downstream reaches where *Cladophora* was more abundant during summer 2012 (Table 3). Differences between upstream and downstream reaches, however, were small. Consumer niche breadths displayed no longitudinal gradient during autumn 2013 when *Cladophora* was less abundant in all reaches (Table 6). Niche breadth was also smaller in autumn 2013 despite a wider range in sampled food resource $\delta^{13}C$ values. Reasons for this are unknown, though more replication of consumers and some food resources may have increased precision of data.

Longitudinal trends: consumer diets

This study addressed whether increased *Cladophora* availability in downstream reaches was correlated with increased assimilation of this macroalgae by primary consumers. Stable isotope analyses during summer 2012 and autumn 2013, however, suggested that primary consumer diets did not reflect differences in *Cladophora* availability.

Cladophora and *P. ceratophyllum* availability were considerably different between the two years, most likely due to differences in hydrological variability. *Cladophora* is susceptible to scouring, with most growth and biomass accrual typically occurring during periods of low water (Power 1990; Ensmingner et al. 2000; Power et al. 2009). Summer 2012 was characterized by stable, low flow discharge between June and August (Figure 2). Consequently, *Cladophora* accrual was high, especially in

downstream reaches where it visibly covered a large portion of the river channel (Figure 5). Alternatively, summer and autumn 2013 were characterized by overall higher discharge rates and more frequent spates (Figure 2). While *Cladophora* accrual was highest downstream, mean cover ranged only between 0 and 1.4% in the four study reaches. *Cladophora* could be found in low levels all reaches, especially along banks and in areas protected from scouring, but was not an abundant food resource. *Podostemum ceratophyllum* cover also showed differing longitudinal trends between the two study years. *Podostemum ceratophyllum* displayed inter-reach variation during summer 2012 but did not show a longitudinal gradient. During autumn 2013, however, *P. ceratophyllum* standing stocks were higher in upstream reaches (Figure 4). Unlike *Cladophora*, *P. ceratophyllum* is more resistant to scouring and has holdfasts that anchor it to the substrate (Hill & Webster 1982b; 1984). High water periods preceding sampling may have led to bedload movement and burial of *P. ceratophyllum* in downstream reaches.

Results from IsoSource analyses suggested that consumers assimilated similar amounts of *Cladophora* in all reaches. This pattern was found in both years (Figures 8-30, 48-61). Additionally, IsoSource models that included *P. ceratophyllum* suggested that *Cladophora* was not a prominent food resource for most consumers (Figures 8-30, 48-61). This contrasts with other food web studies in this river. Stable isotope research in prior years has shown that *Cladophora* is a prominent food resource in the furthest downstream reach during summer and autumn (Tinsley 2012, Yates 2012).

One potential explanation is that consumer isotopes were influenced by diets when *Cladophora* was relatively scarce. Although *Cladophora* levels were relatively

high when consumers were sampled in 2012 (Figure 5), consumer isotopic values were likely also influenced by diets earlier in the spring. Algal $\delta^{13}C$ values often change seasonally and consumer isotopes change slower than their food resources (Fry & Arnold 1982; Hesslein et al. 1993; Cabana & Rasmussen 1996). Consumer stable isotope samples were taken within a few weeks of the onset of low-flow conditions and the subsequent *Cladophora* bloom during summer 2012 (Figures 2, 5). Highly ^{13}C-depleted values of some consumers relative to sampled food sources during summer 2012 provide evidence that consumer isotopes may still be influenced by diets earlier in the spring when *Cladophora* was essentially not yet available as a food resource. Although food resources were not sampled during early spring due to flow conditions that did not permit wading, $\delta^{13}C$ values of algae in other streams are known to be more ^{13}C-depleted during winter and early spring compared to later seasons (McCutcheon & Lewis 2002; Vizzini & Mazzola 2003).

If consumer isotopes reflect food resources assimilated earlier in the spring in 2012, then isotope samples likely reflect diets during seasons when *Cladophora* was not extremely abundant at any reach. *Cladophora* typically is scarce during winter and early spring high flows, then rapidly can become abundant during warmer low-flow seasons (Power et al. 2009; Penick et al. 2012). Sampling occurred later in the season during autumn 2013 when *Cladophora* is typically abundant (Penick et al. 2012), but frequent spates prevented higher *Cladophora* accrual (Figure 2, 5). While consumers do not appear to differentially assimilate *Cladophora* in downstream reaches during spring 2012 and autumn 2013, food web analyses during more stable *Cladophora* blooms may still find increased reliance of *Cladophora* downstream.

Consumer diets

Longitudinal gradients in consumer $\delta^{13}C$ values matched patterns within in-stream primary producer stable isotopes, suggesting that consumers during both years assimilated autochthonous food sources (Figures 6, 46). Other studies have demonstrated that consumers display similar $\delta^{13}C$ and $\delta^{15}N$ patterns as their algal food resources (Finlay 2001; McCutcheon & Lewis 2002). These results are not surprising since food webs in mid-order streams are typically based on in stream primary producers (Vannote et al. 1980; Rosi-Marshall & Wallace 2002; Leberfinger et al. 2011).

What is unclear, however, is whether consumers were assimilating *P. ceratophyllum*, *Cladophora*, their associated epiphytes, or a combination of epiphytes plus the plant host tissues. IsoSource analyses of the 2012 data yielded conflicting results. Analyses that included *P. ceratophyllum* typically had much higher source contributions for this macrophyte than for *Cladophora* (Figures 8-20). *Podostemum ceratophyllum*, however, has lower nutritional quality and is not typically assimilated by primary consumers (Hutchens et al. 2004; Tinsley 2012). In contrast, analyses that did not include *P. ceratophyllum* often did not have feasible source contributions because consumers were more $\delta^{13}C$-depleted than food resources. Results from 2013 present a similar dilemma. Longitudinal patterns in consumer $\delta^{13}C$ values matched trends within *P. ceratophyllum* but not *Cladophora* (Figure 46).

Alternatively, *Cladophora* cannot be ruled out as a feasible resource for primary consumers during either year. Consumer isotopes reflect long-term mean $\delta^{13}C$ values of food resources (Fry & Arnold 1982; Hesslein et al. 1993). *Cladophora* is fast growing,

yet frequent spates, which occurred earlier during May 2012 and August 2013, can remove large proportions of algal biomass (Dodds & Gudder 1992). Current *Cladophora* $\delta^{13}C$ values are likely to reflect more short-term geochemical conditions. *Podostemum ceratophyllum*, however, is a slower growing macrophyte that is less susceptible to scouring (Hill & Webster 1982b, 1984), and consequently, $\delta^{13}C$ values are more likely to reflect longer-term macrophyte $\delta^{13}C$ value averages. Singer et al. (2005) suggested that weekly samplings of algal resources under some growth conditions may be necessary to find average $\delta^{13}C$ values of resources. Because algal resources have a much higher turnover rate than consumer isotopes, samples of *Cladophora* likely did not have $\delta^{13}C$ values representative of overall average *Cladophora* isotopic values throughout the season. Furthermore, this taxon is more likely to be a nutritious food resource. Unlike *P. ceratophyllum*, several studies have concluded that *Cladophora* can be directly grazed by consumers (Rhame & Stewart 1976; Marcus et al. 1978; Gray & Ward 1979; McShaffrey & McCafferty 1991; Dodds & Gudder 1992).

A third potential algal-based food resource is epiphytes. Several studies have noted that *Cladophora* and *P. ceratophyllum* provide substrate for labile epiphytes (Dodds 1991; Hutchens et al. 2004; Roll et al. 2005; Furey et al. 2012). Epiphytes were not isolated from either *Cladophora* or *P. ceratophyllum*, yet samples of both macroproducers had dense epiphytic growth clearly visible under light microscopy during both sampling periods. Additionally, a chironomid larva was observed feeding on *Cladophora* epiphytes under a dissection scope. Epiphytes were scraped off but only *Cladophora* fragments were seen passing through gut tract unchanged.

Despite strong evidence for consumption of autochthonous resources, mixing model analyses also suggest that consumers also rely partially on detrital resources. IsoSource results for consumers during summer 2012 suggested that macroinvertebrates assimilated terrestrial leaves (Figures 8-30). Although dietary analyses were less clear during autumn 2013, mixing polygons and IsoSource analyses suggested that consumer stable isotopes could not be explained solely by *Cladophora* or *P. ceratophyllum*. IsoSource mixing models suggested that consumers assimilated several food resources, including leaves and other detrital resources (Figures 48-61). While many current studies emphasize the role of algal food resources in food webs, this study provides some evidence that detrital inputs may remain an important, albeit secondary, role within primary consumer diets in midreach systems (Vannote et al. 1980; Mayer & Likens 1987; Delong & Thorp 2006; McNeely et al. 2007).

Mixing model results in 2013 had less precise results than 2012 results. This was surprising, given increased replication of both food resources and consumers during the latter sampling period. One potential contributing factor is the presence of several resources that had similar isotopic values during autumn 2013 (Figure 46). Mixing model results may have less resolution when food resource isotopic values overlap or where consumers are at the center of the mixing polygon (Fry 2007; Winemiller et al. 2007). Food resource availability may also be a contributing factor to IsoSource variability during 2013. Resources such as *Cladophora* and in downstream reaches, *P. ceratophyllum*, had decreased cover during autumn 2013 presumably because of more frequent spates (Figure 2, 4-5). Variable hydrology during the sampling period may have

decreased the abundance of previously abundant food resources, and consequently, primary consumers may have increased consumption of alternative food sources.

Consumers are expected to assimilate largely autochthonous resources in midreaches (Vannote et al. 1980). Although consumer isotopes reflected diets during lower *Cladophora* growth, results suggested that they still relied, at least partially, on autochthonous food resources. Allochthonous resources, especially terrestrial leaves, may also provide a secondary food source in midreaches during time periods when algal food resources are less abundant.

Summary

No definitive cause can be identified for longitudinal gradients in producer and consumer $\delta^{13}C$ values. One potential explanation, however, is that carbon geology may modify normal longitudinal gradients in consumer and algal $\delta^{13}C$ values by increasing DIC availability. DIC is usually less available in midreaches compared with headwaters, but in a previous study of the Green River, DIC was more abundant in more karstified downstream reaches (Osterhoudt 2014). If this pattern persists, increasing DIC abundances downstream may cause in-stream primary producers and consumers to become more ^{13}C-depleted .

Although underlying changes in geology coincided with increased *Cladophora* availability and longitudinal gradients in producer and consumer $\delta^{13}C$ values, consumer diets and niche breadth did not appear to differ between reaches. Further testing during periods of high-*Cladophora* abundance, however, is needed to determine whether consumer diets display longitudinal gradients during stable *Cladophora* blooms.

Mixing model results also suggested that primary consumers relied on both autochthonous and allochthonous food resources. Consumers in midreaches are expected to assimilate largely autochthonous resources, but allochthonous resources may remain an important component to stream food webs, especially during periods with lower abundances of algae.

FIGURES AND TABLES

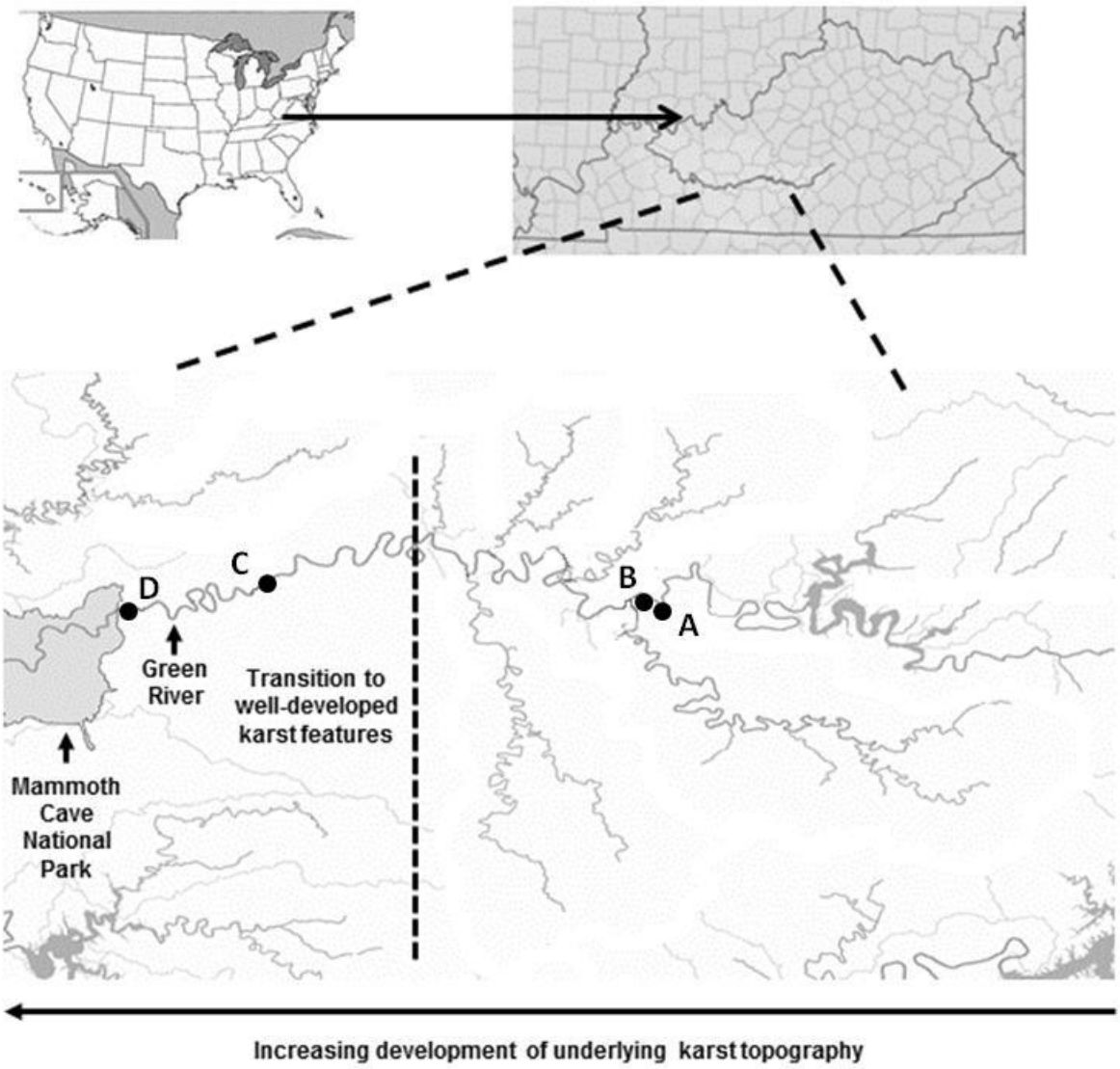

Figure 1. Map depicting the location of the upper Green River and Green River Lake, KY, USA. Sampling reaches are marked by capital letters and solid black circles. Reaches are labeled A through D, with A as the furthest upstream reach.

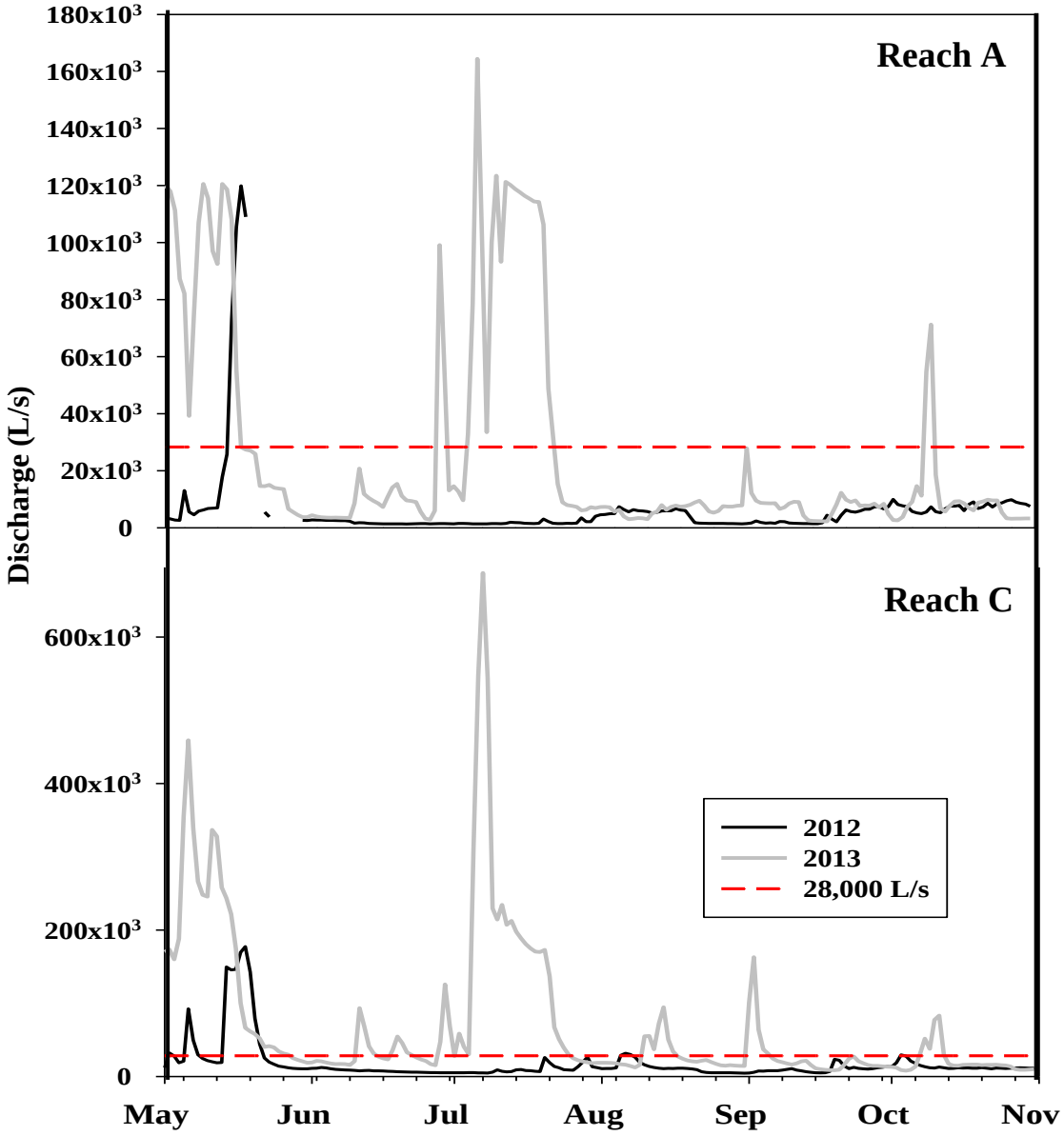

Figure 2: Discharge rates at two reaches in the upper Green River. The red dashed line denotes maximum wadable discharge rate. Data from USGS station Louisville, KY. Greensburg discharge rate was calculated from gauge height using formula from Osterhoudt (2014).

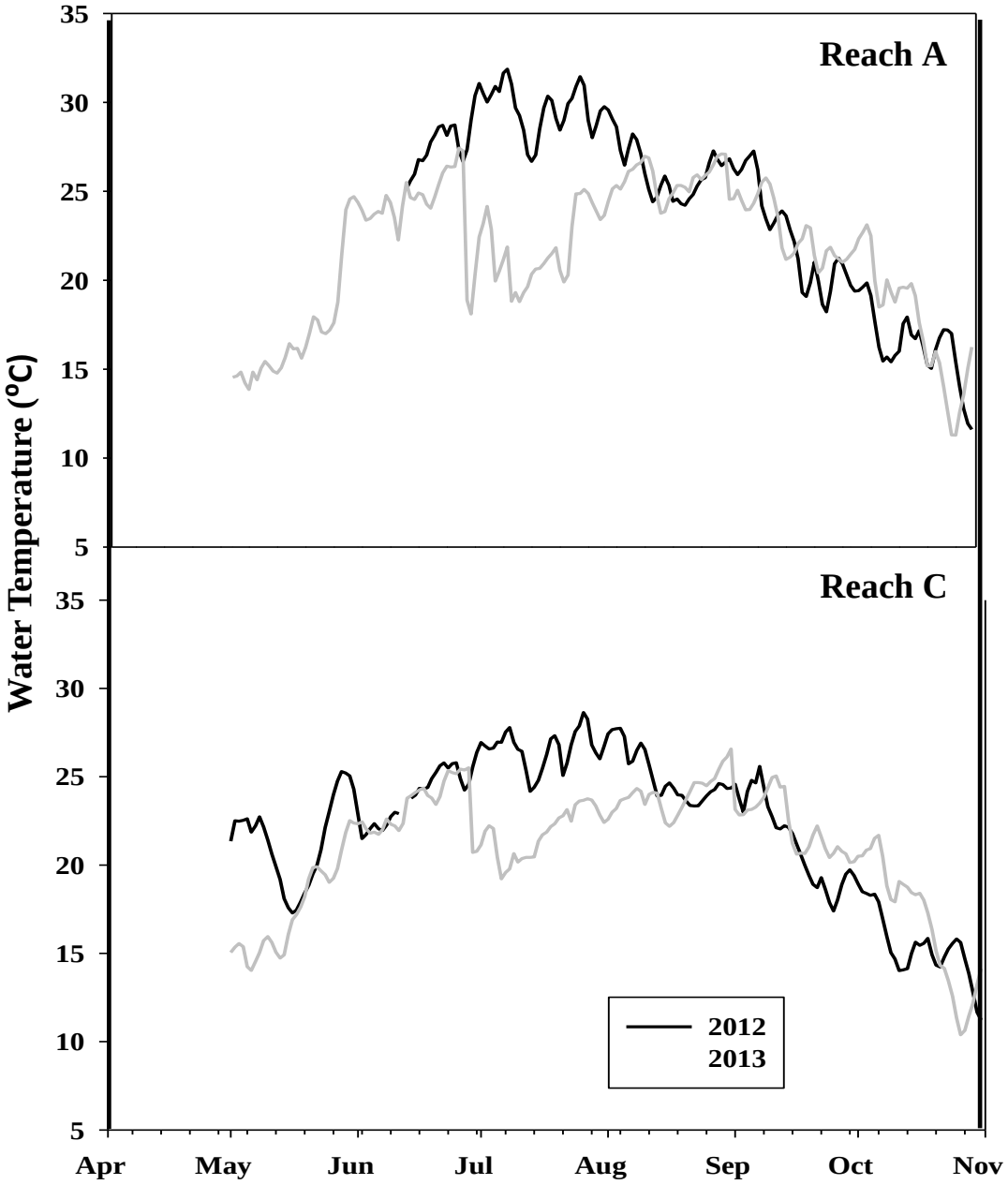

Figure 3: Water temperature in two reaches in the upper Green River. Data from USGS station Louisville, KY.

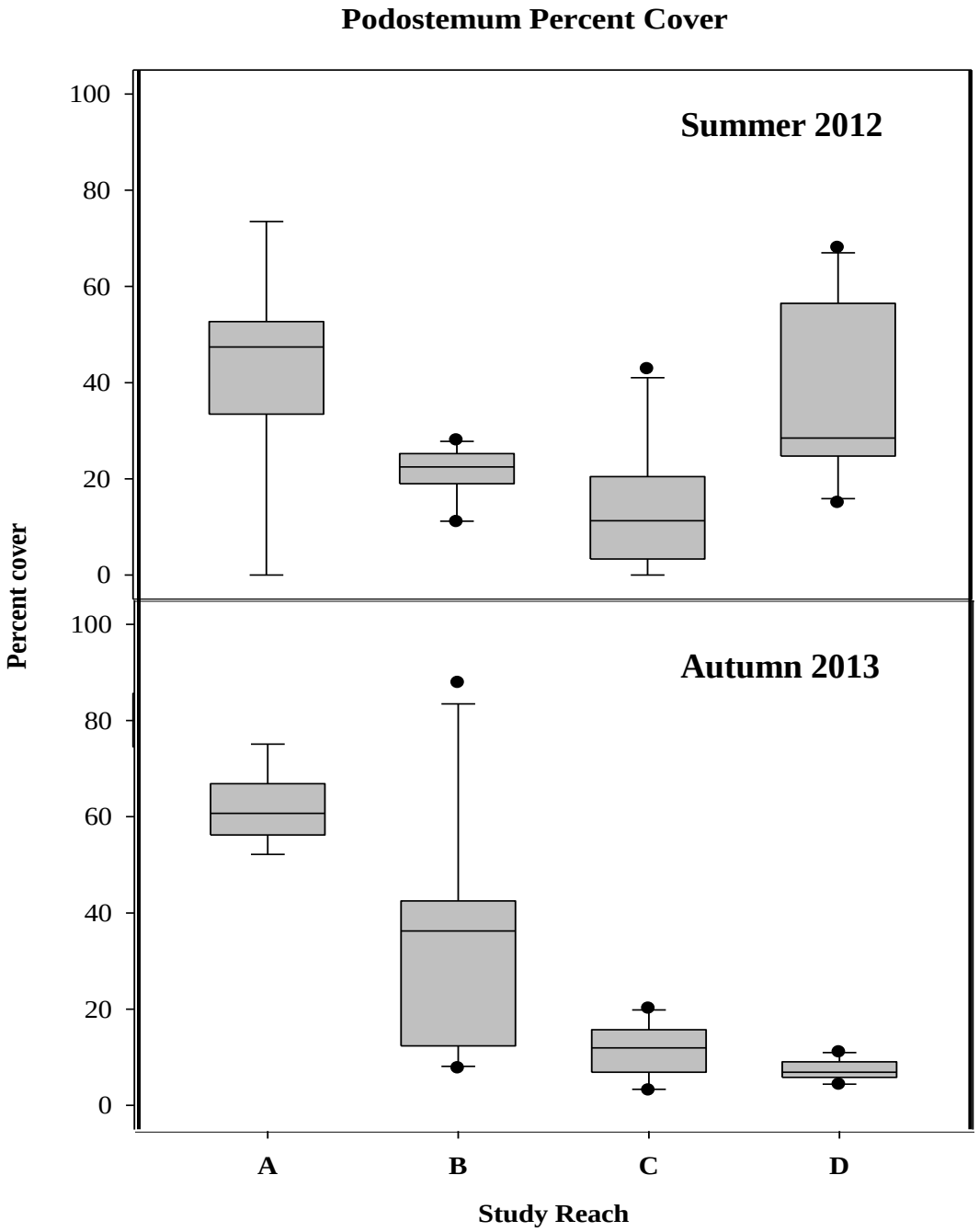

Figure 4: Percent cover for *P. ceratophyllum* at four study reaches along the upper Green River. Boxes extend up to 1 SE and whiskers show 95% confidence interval. Reaches A through D are in order from upstream to downstream.

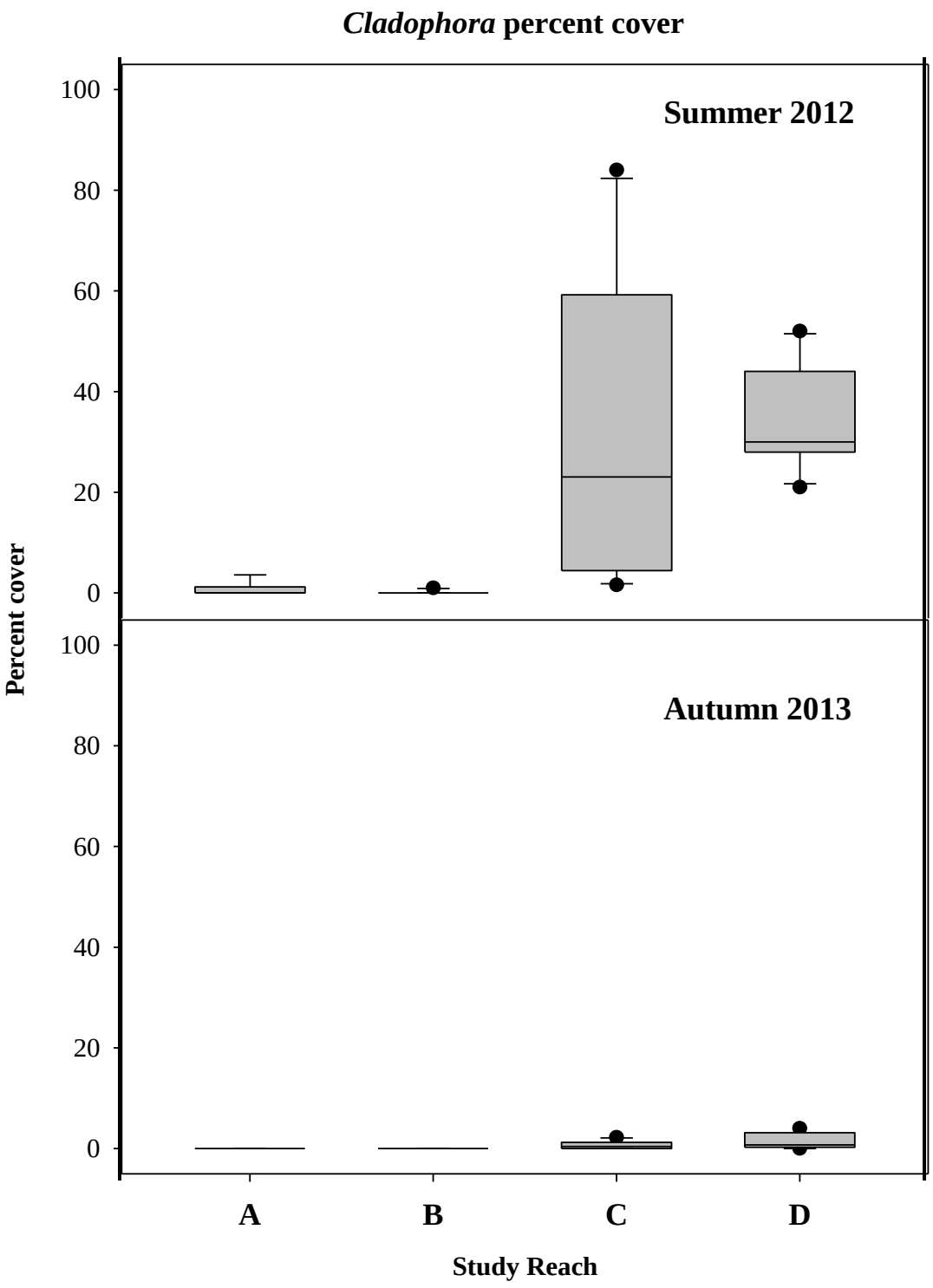

Figure 5: Percent cover for *Cladophora* at four study reaches along the upper Green River. Boxes extend up to 1 SE and whiskers show 95% confidence interval. Reaches A through D are in order from upstream to downstream.

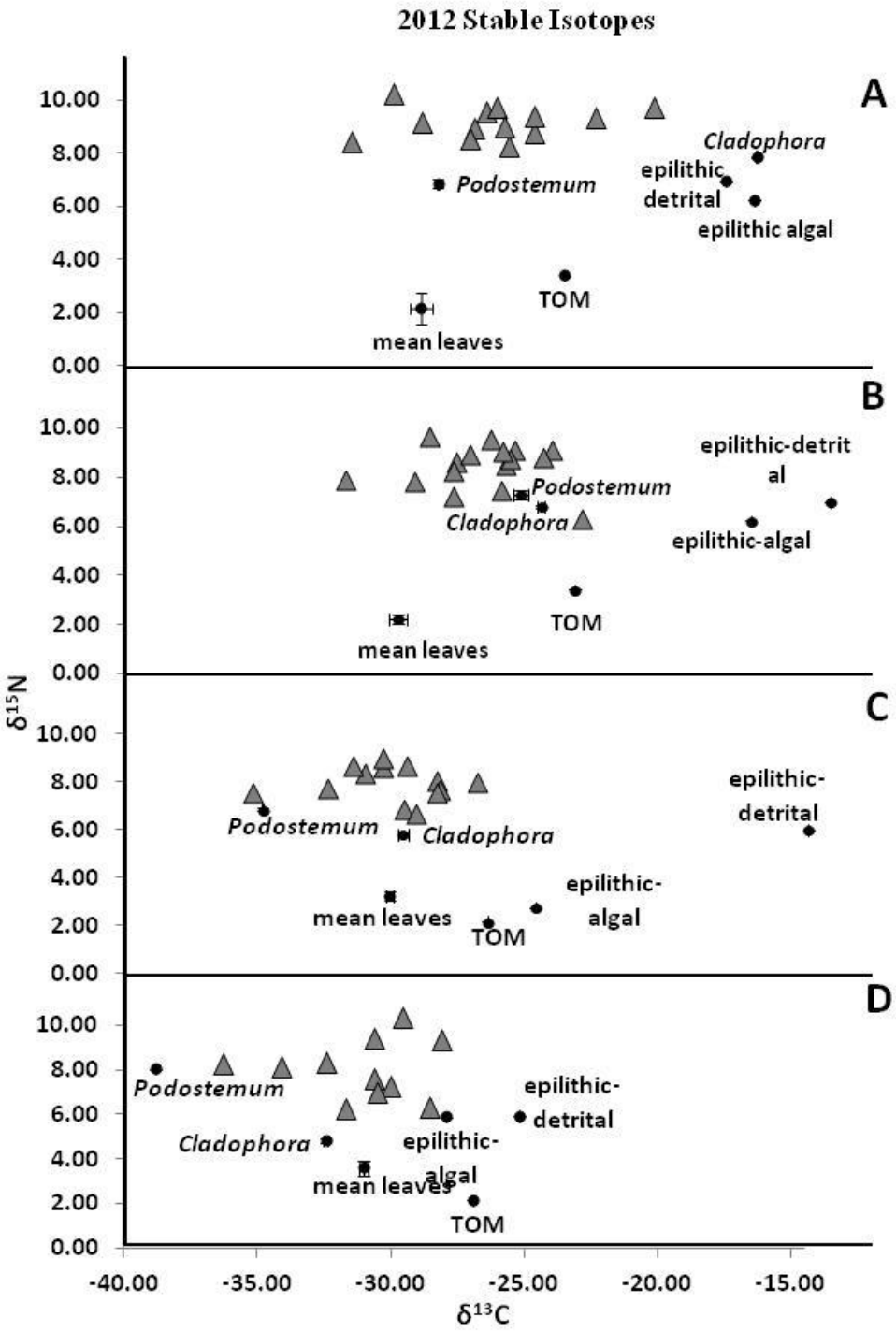

Figure 6: Stable isotope biplot of primary consumers and potential food resources at four reaches along the upper Green River during summer 2012. Potential resources are depicted as circles with ±1SE. Primary consumer taxa are depicted as triangles and represent one sample per taxa. . Reaches A through D are in order from upstream to downstream.

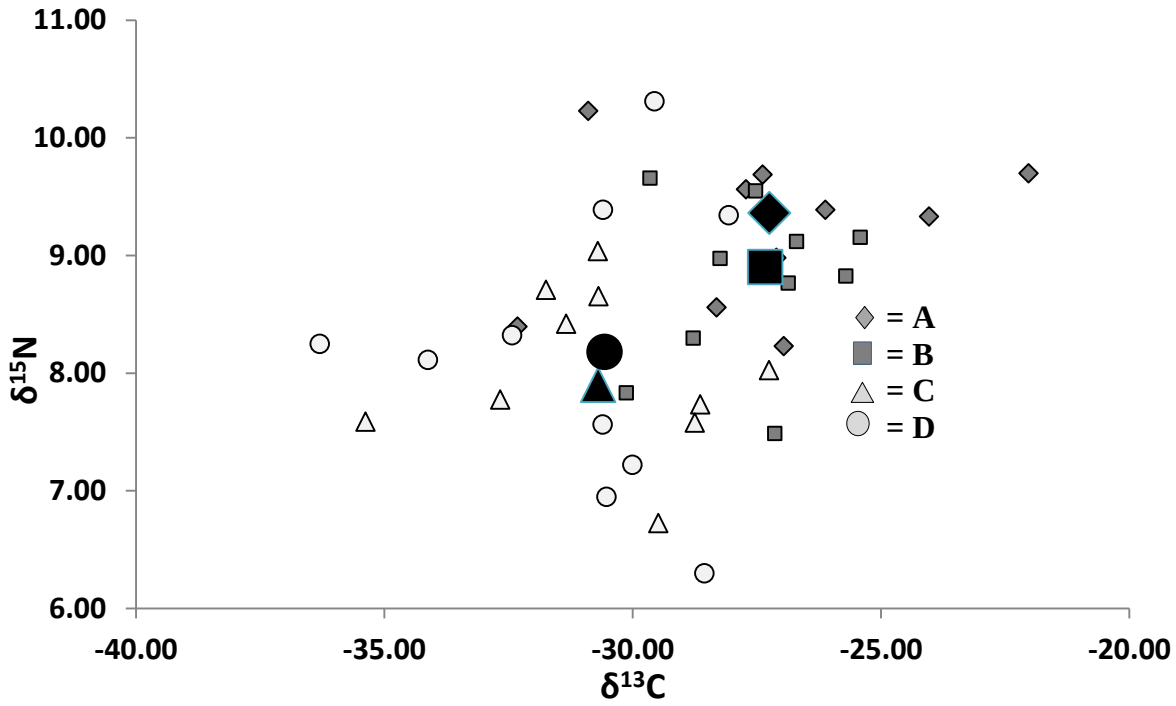

Figure 7: Stable isotope biplot of primary consumers at four study reaches along the Green River during summer 2012. Larger shapes represent median consumer stable isotope values at each reach. Reaches A through D are in order from upstream to downstream.

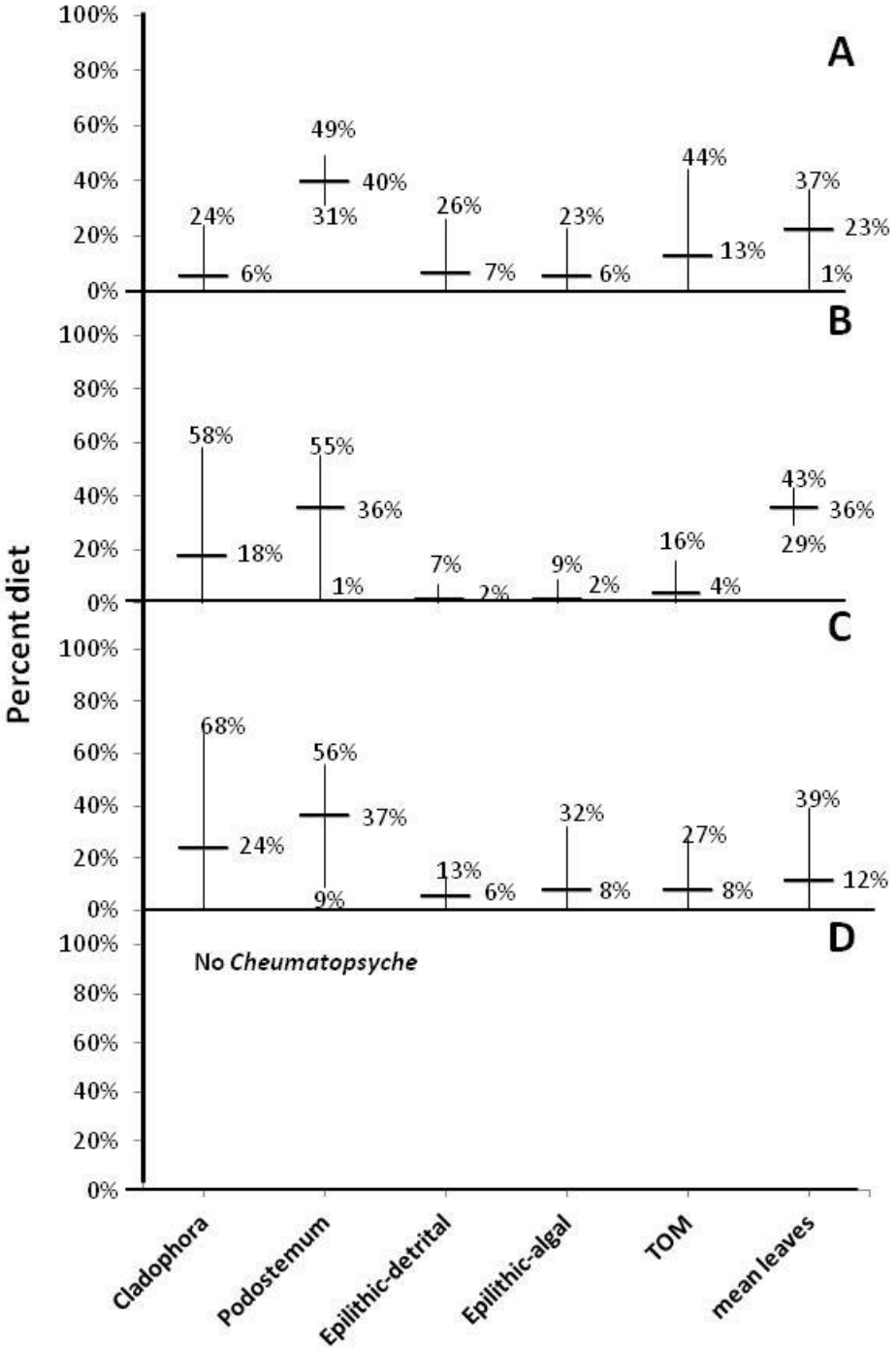

Figure 8: IsoSource results for *Cheumatopsyche* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

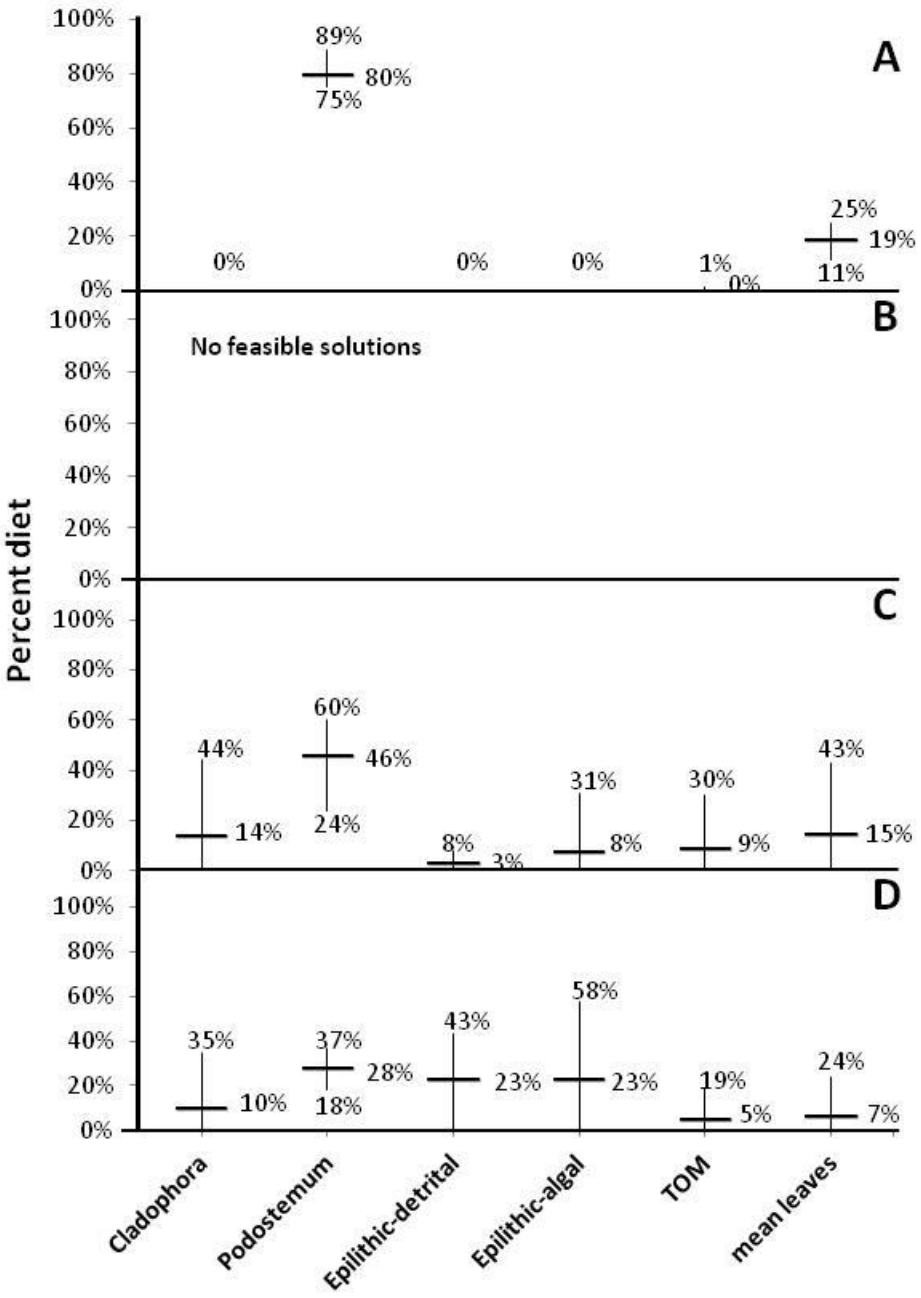

Figure 9: IsoSource results for *C. fluminea* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

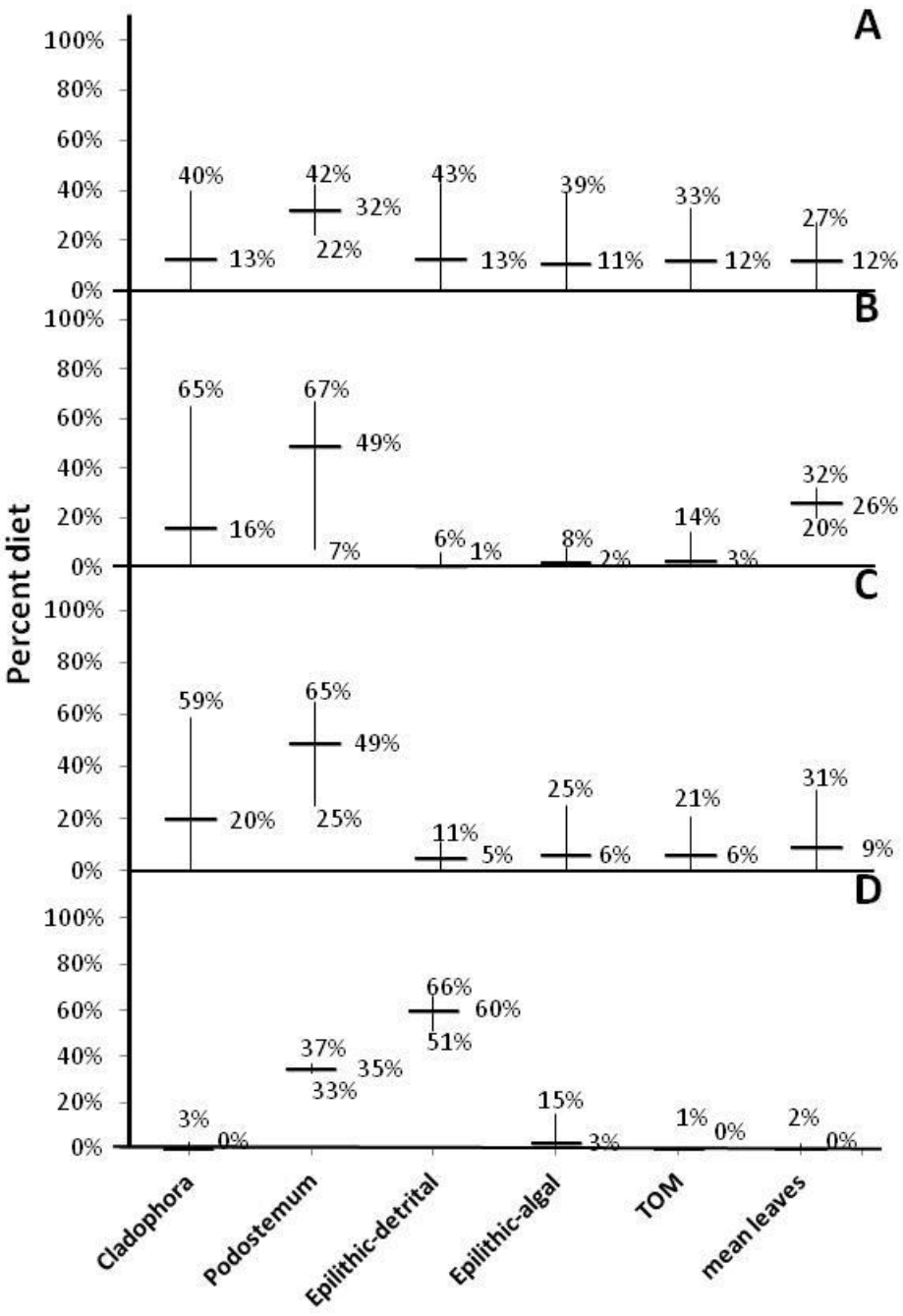

Figure 10: IsoSource results for *H. simulans* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

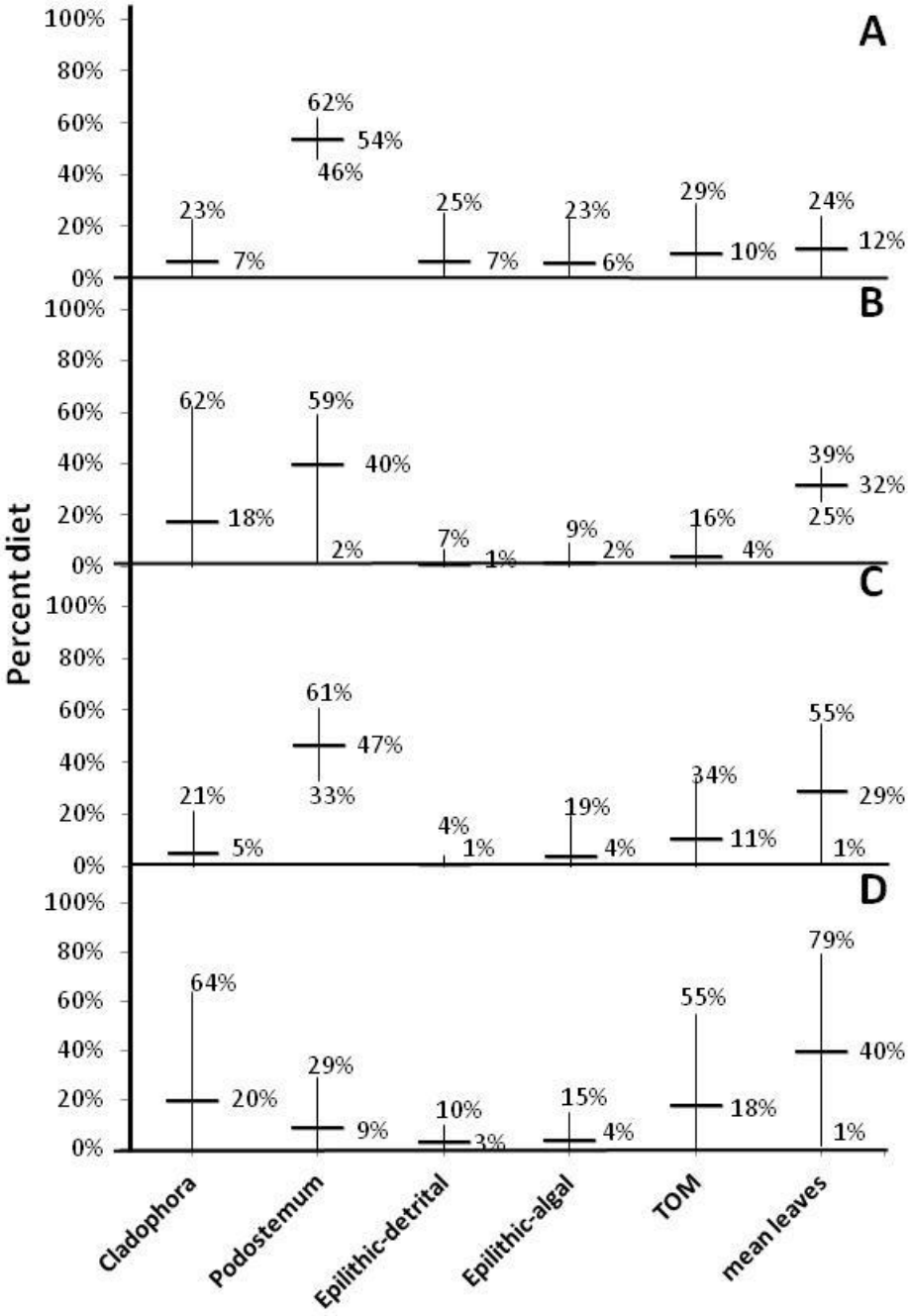

Figure 11: IsoSource results for *Isonychia* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

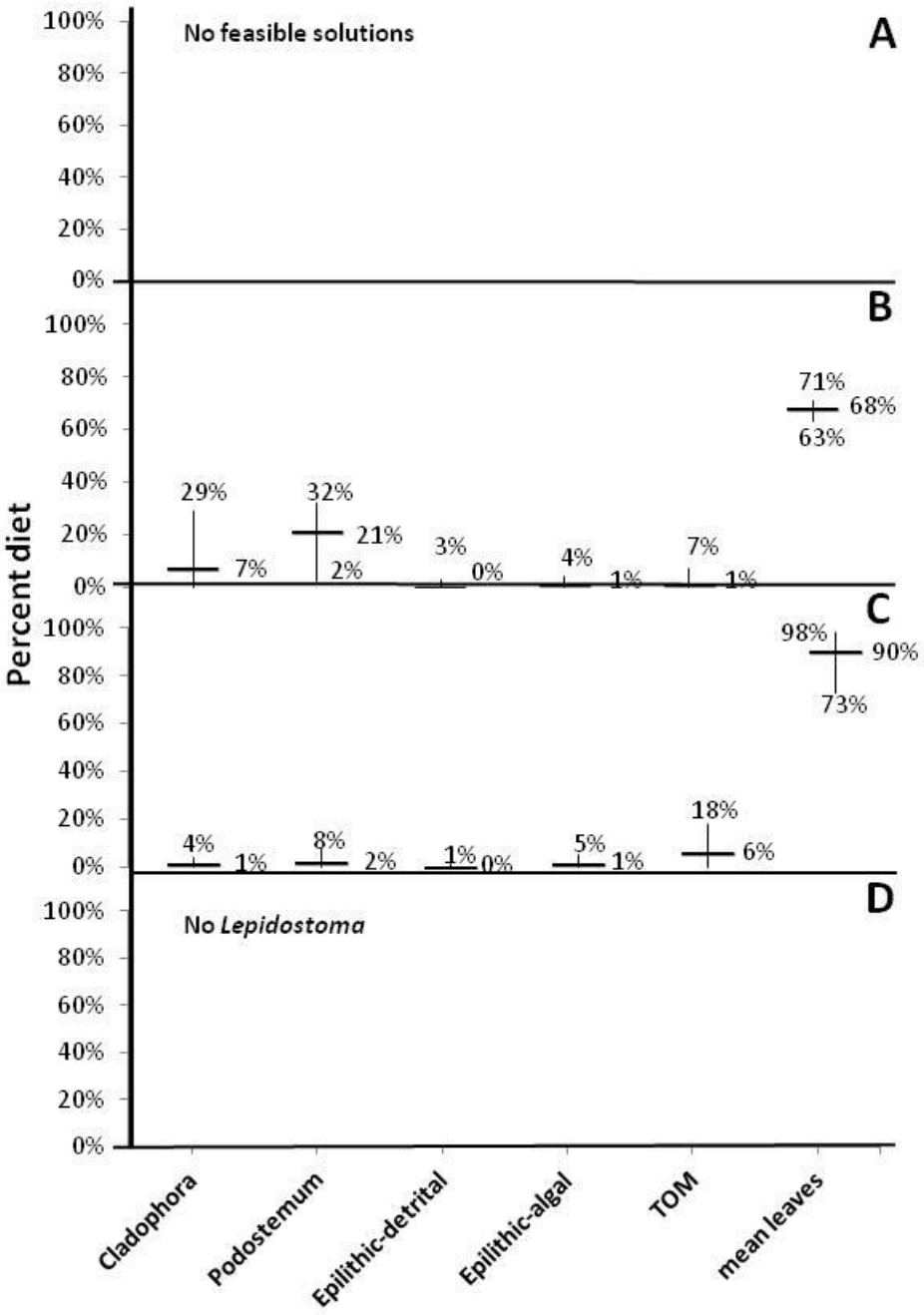

Figure 12: IsoSource results for *Lepidostoma* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

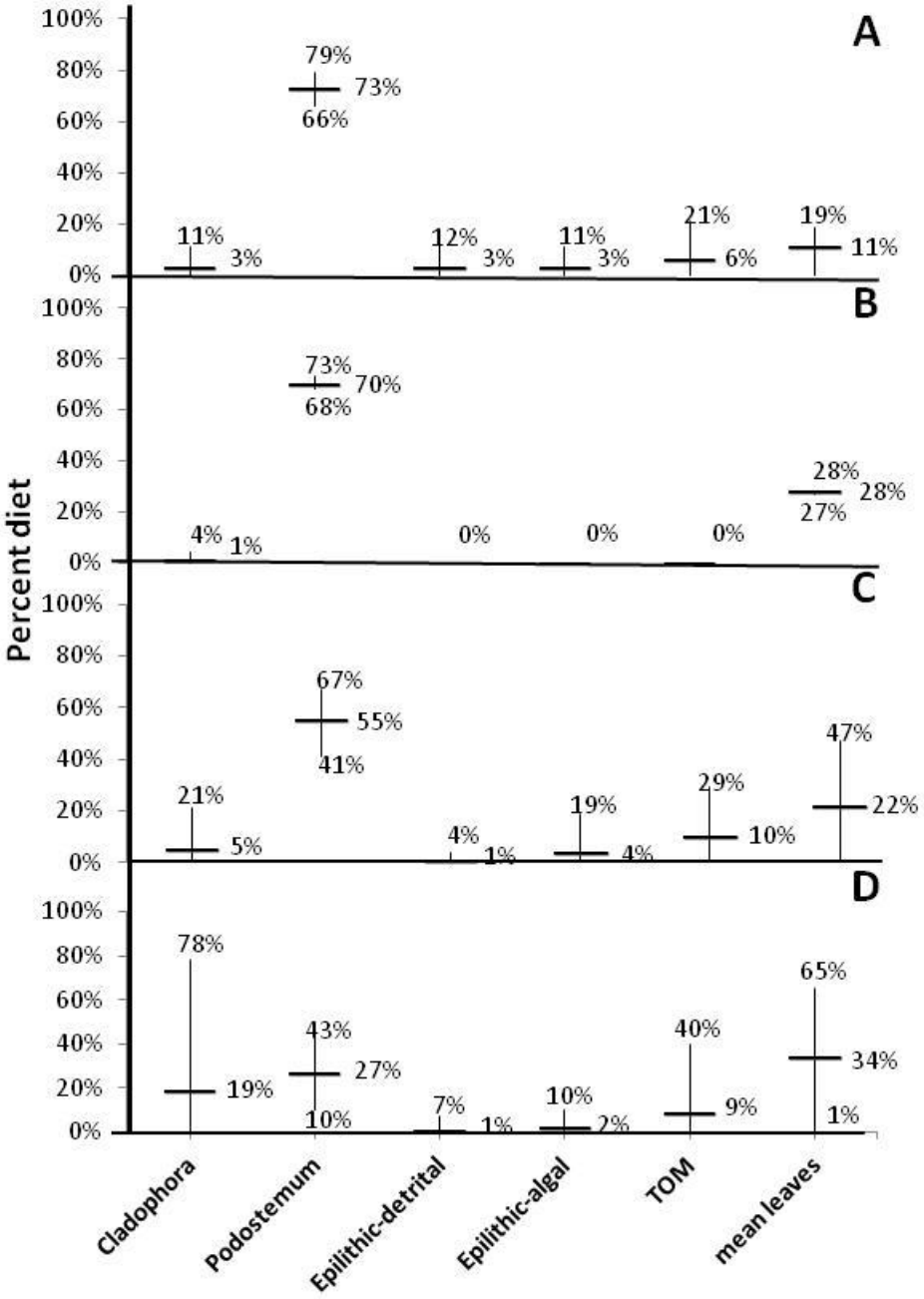

Figure 13: IsoSource results for *L. praerosa* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

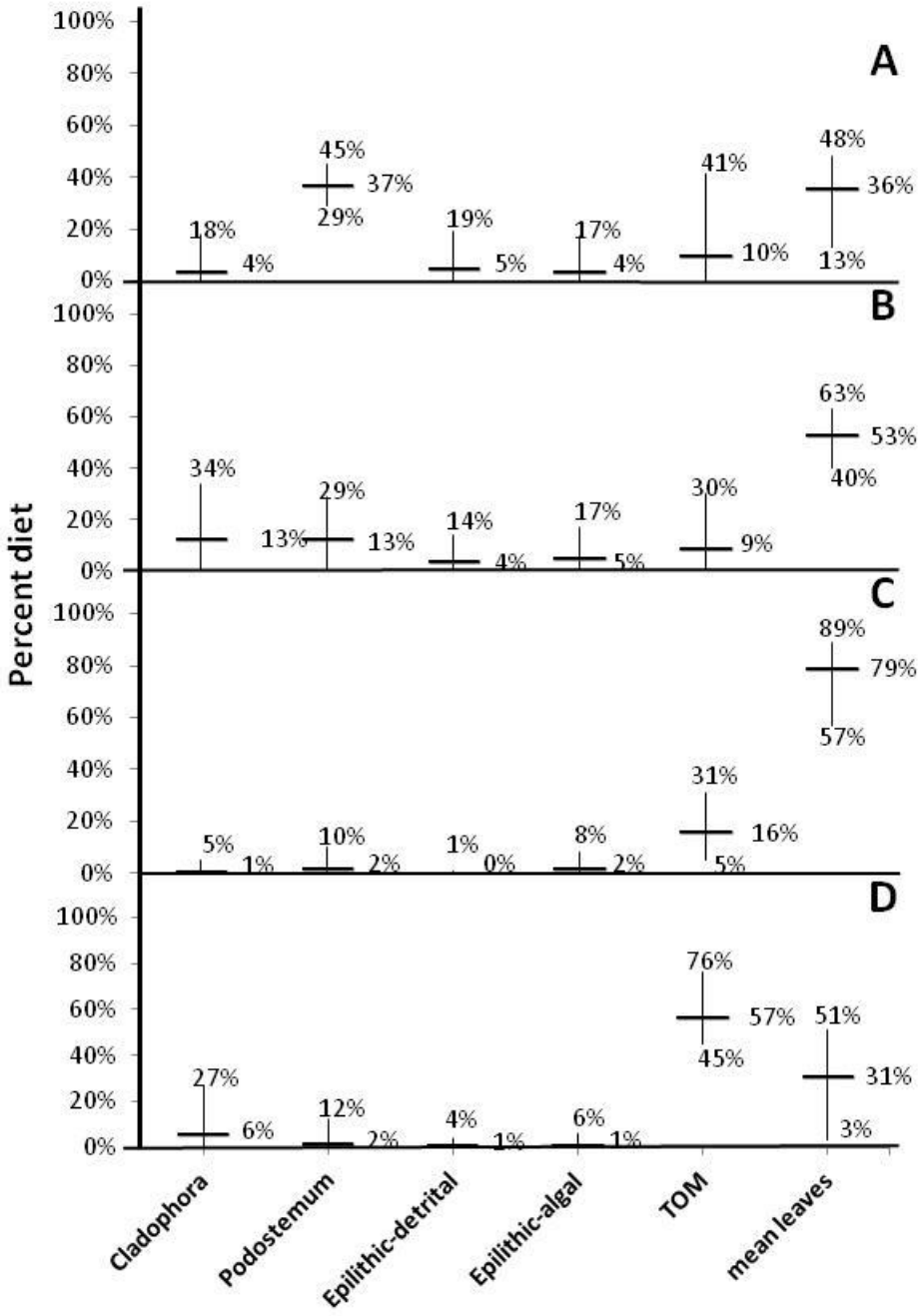

Figure 14: IsoSource results for *Maccaffertium mediopunctatum* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

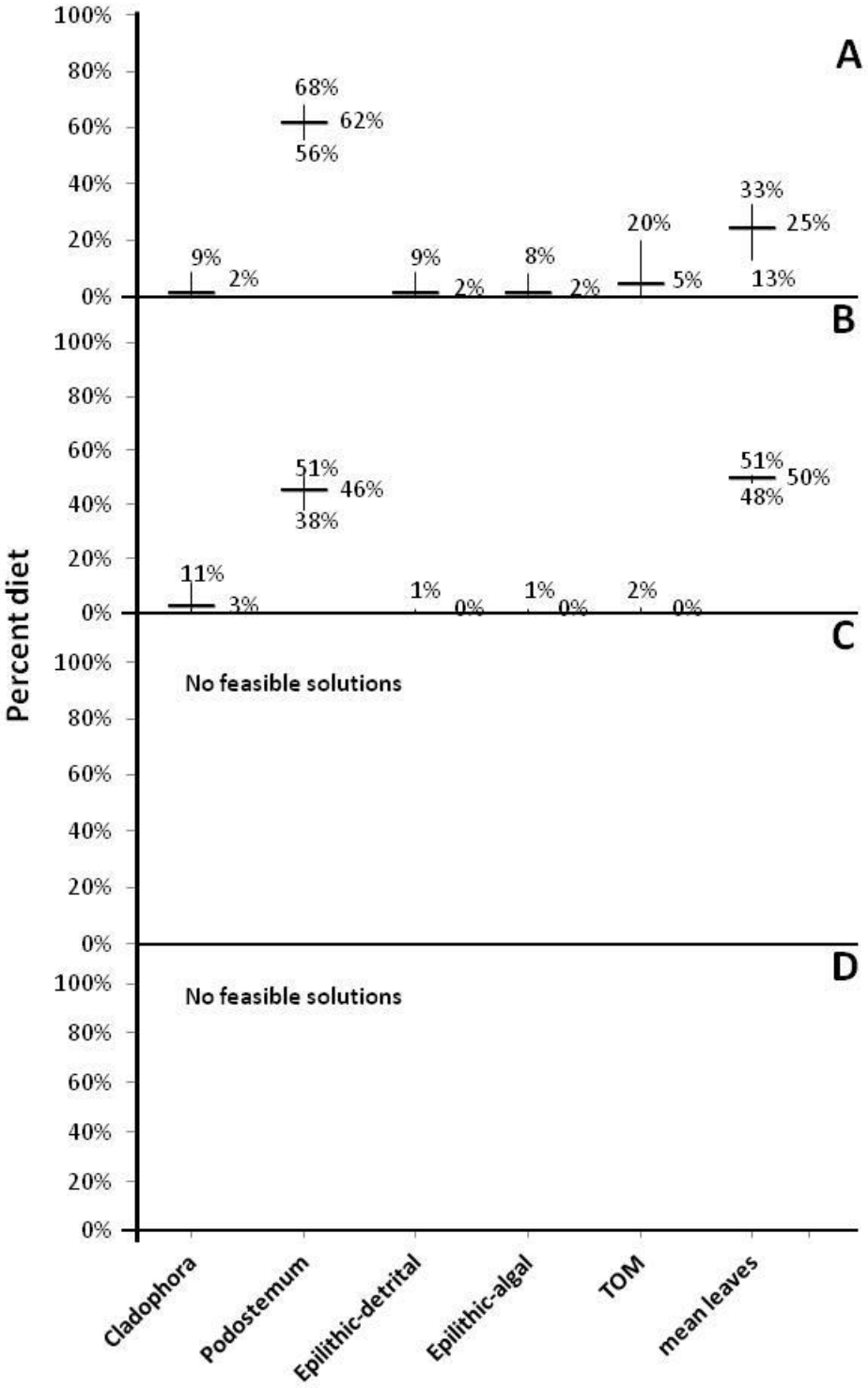

Figure 15: IsoSource results for *Optioservus* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

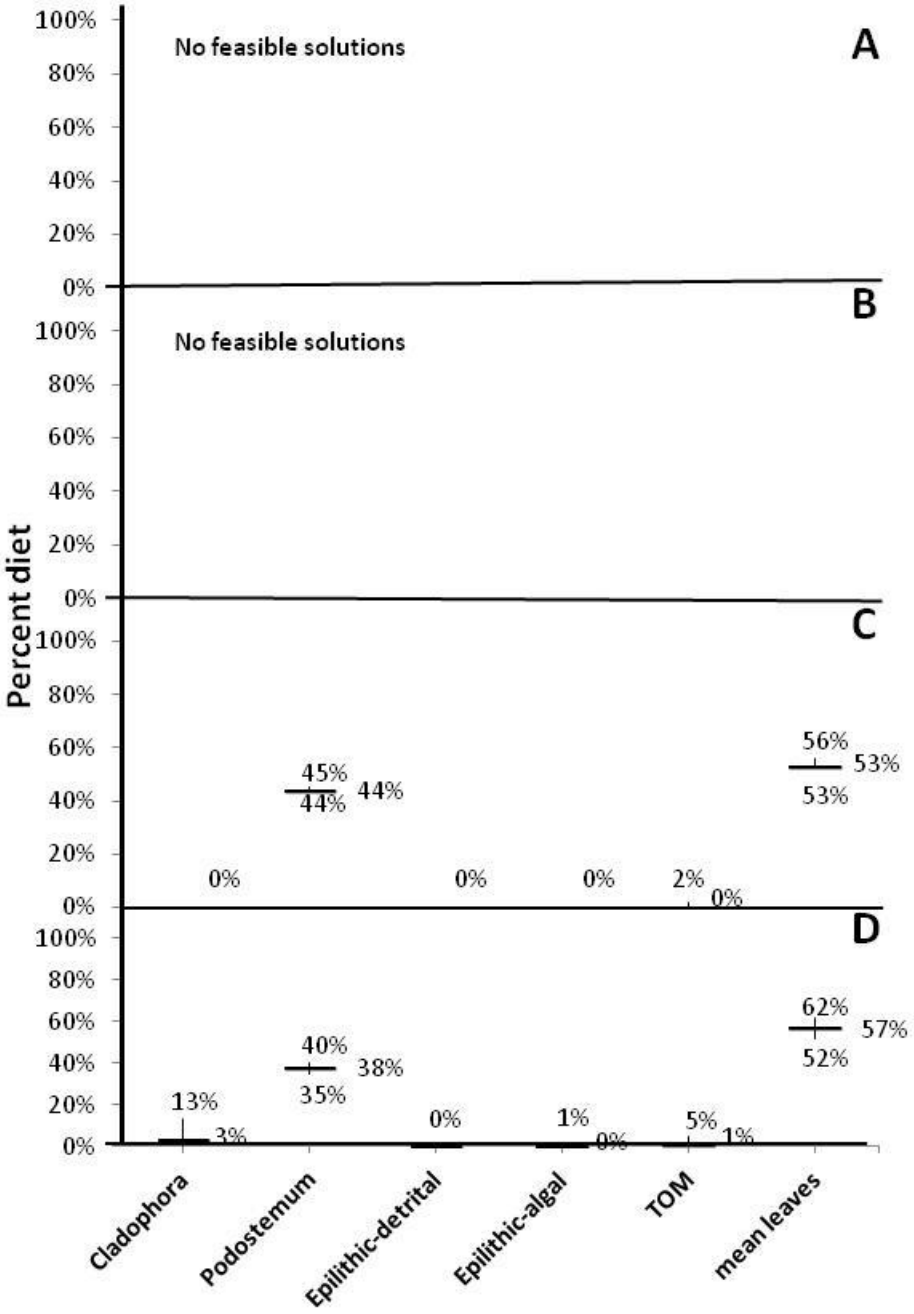

Figure 16: IsoSource results for *P. dorsata* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

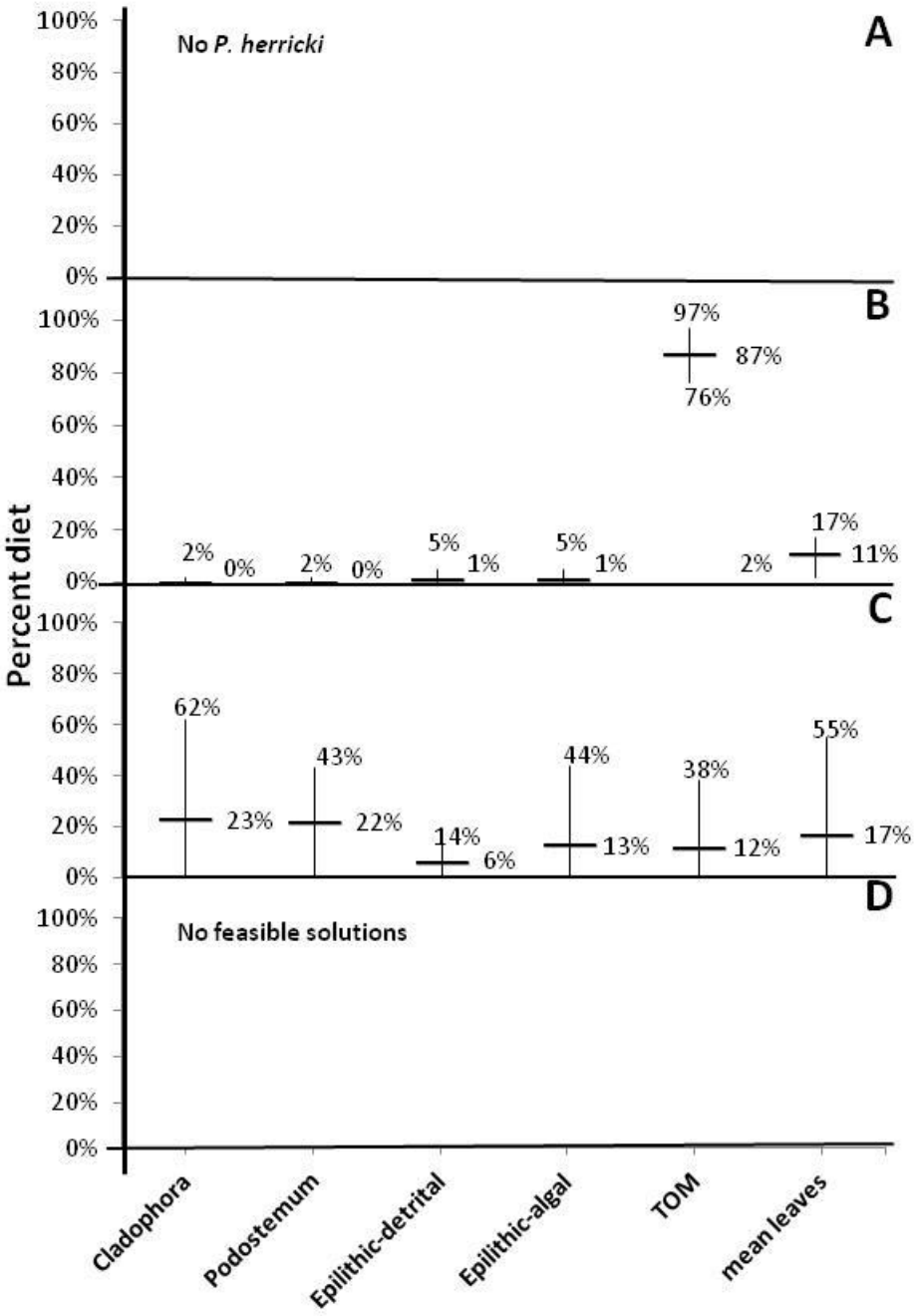

Figure 17: IsoSource results for *P. herricki* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

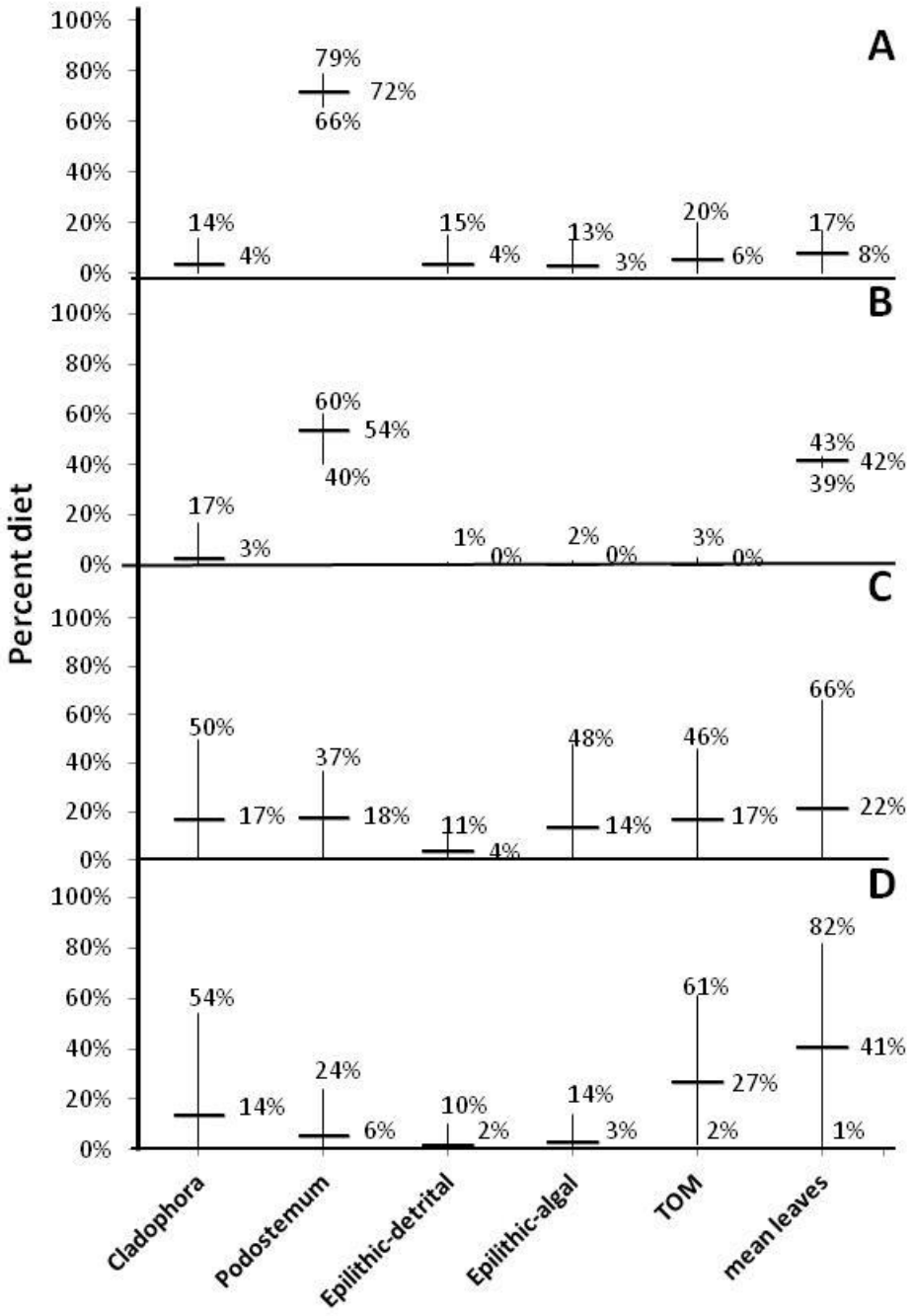

Figure 18: IsoSource results for *Pycnopsyche* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

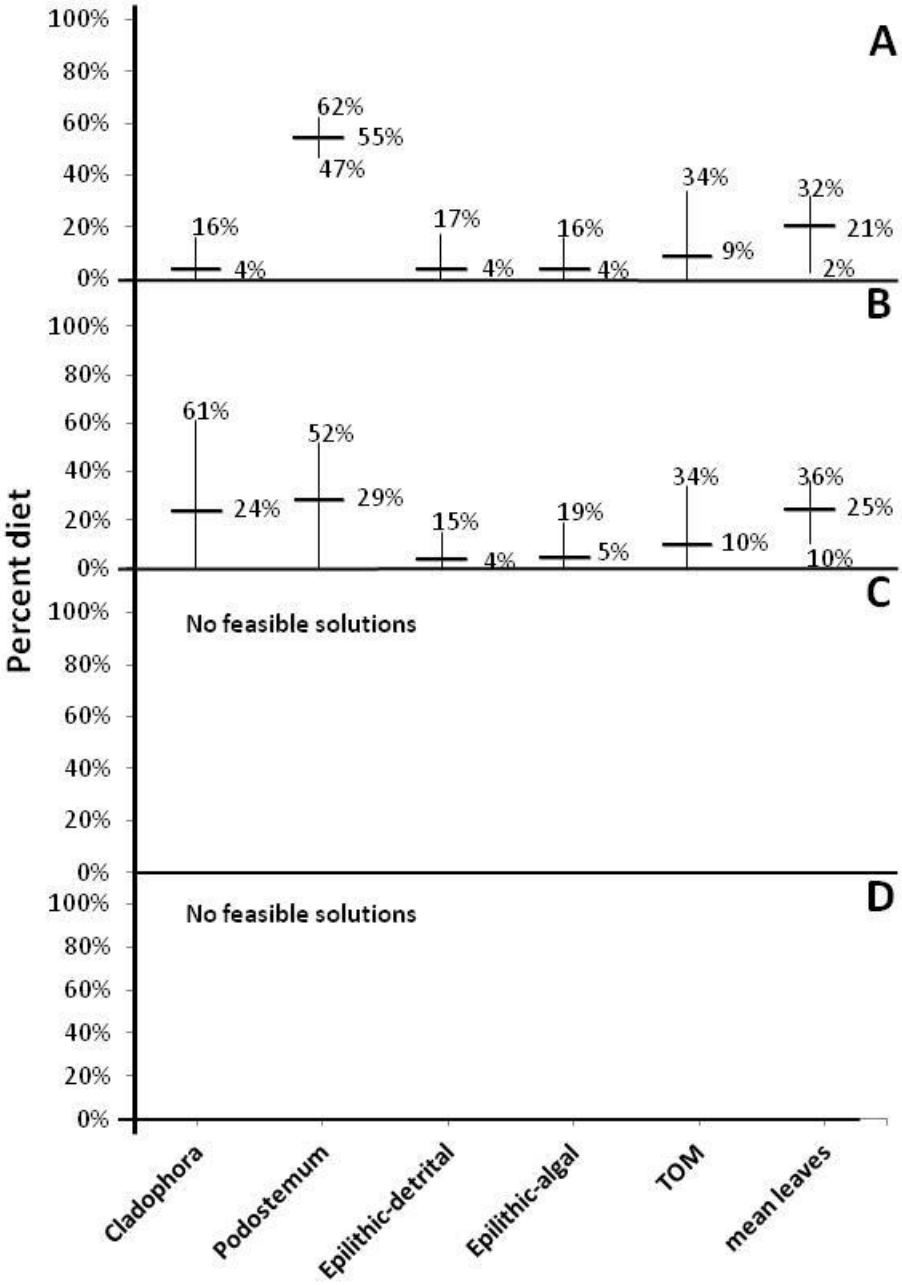

Figure 19: IsoSource results for *Simulium* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

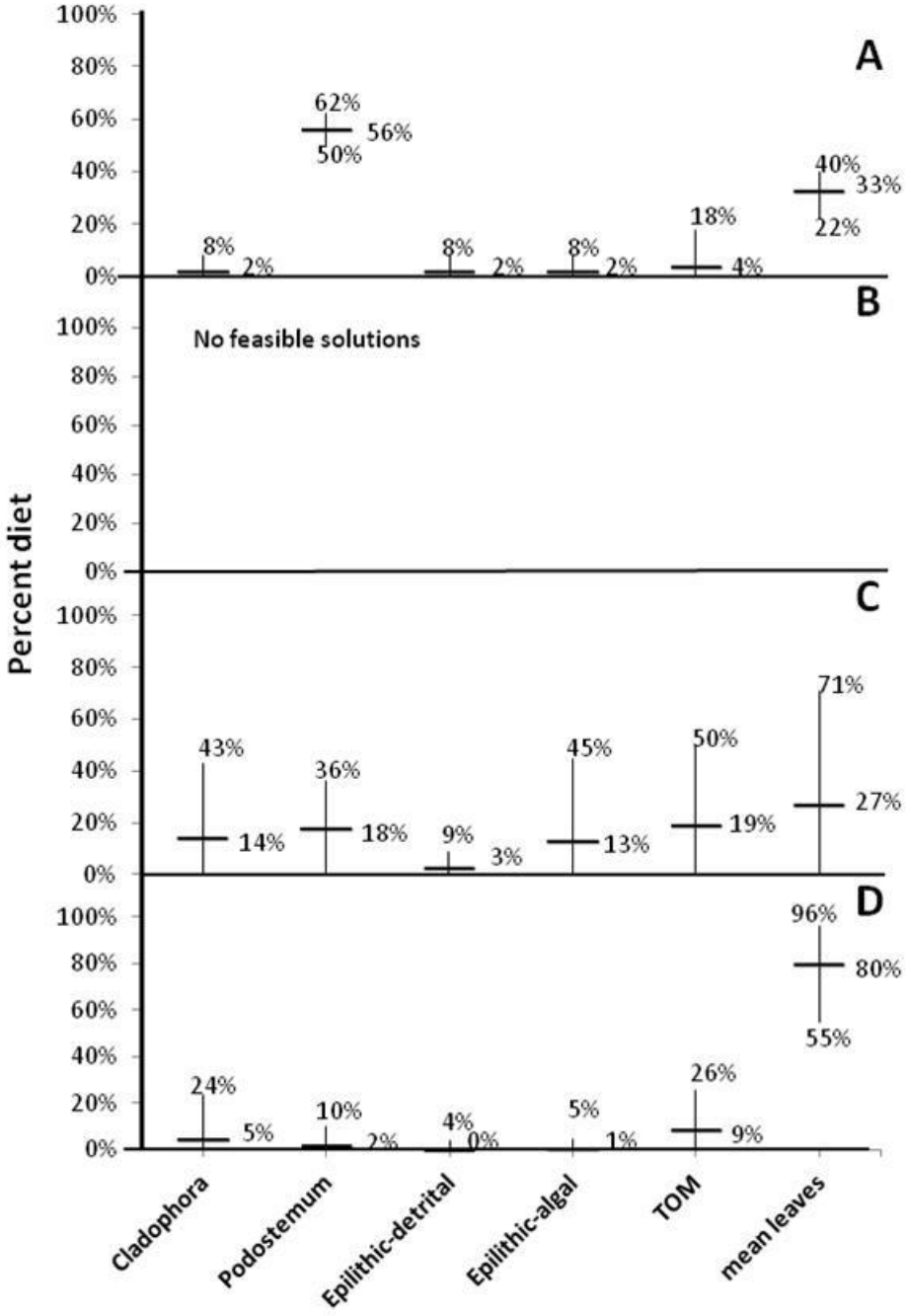

Figure 20: IsoSource results for *S. crenata* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

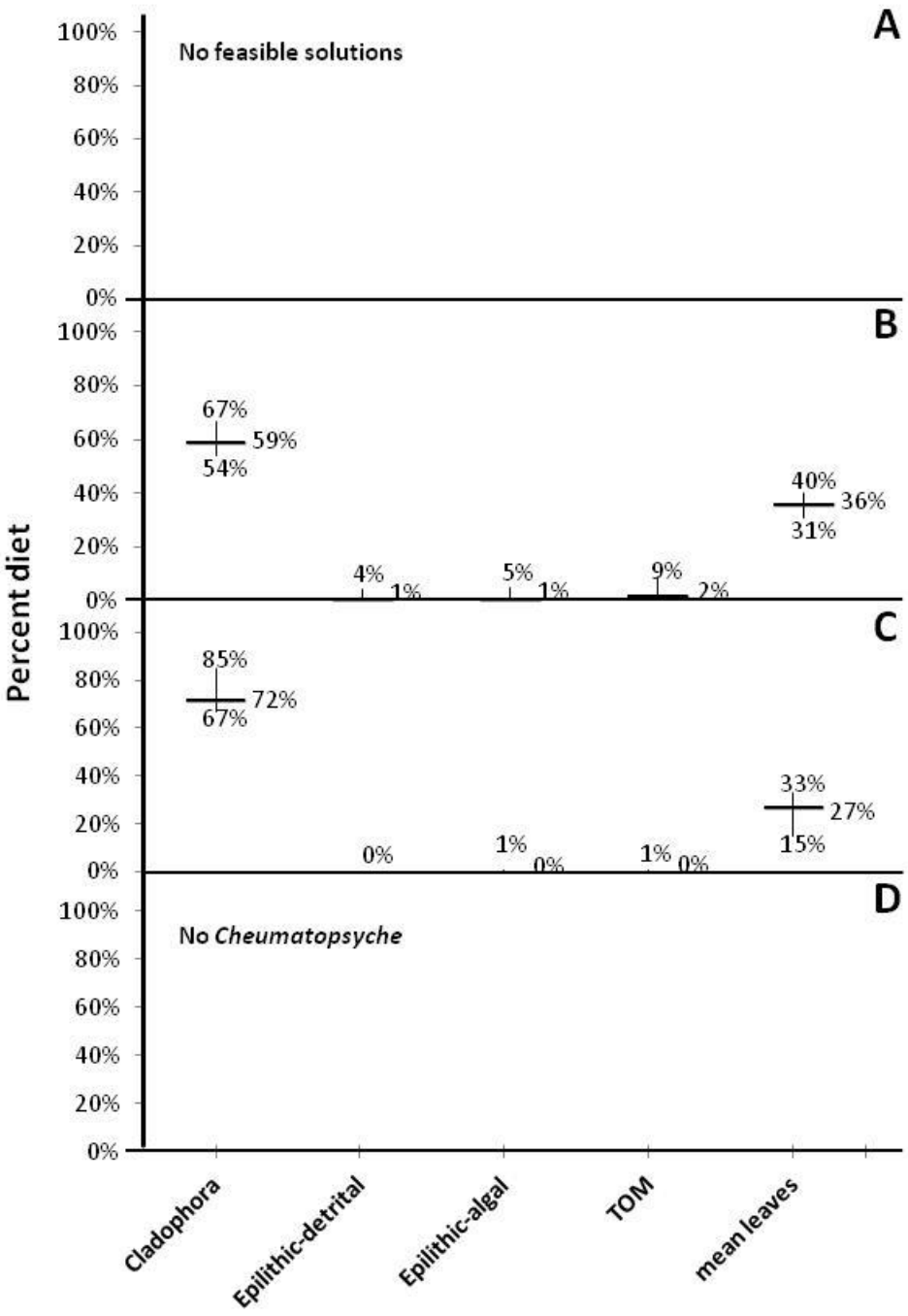

Figure 21: IsoSource results for *Cheumatopsyche* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

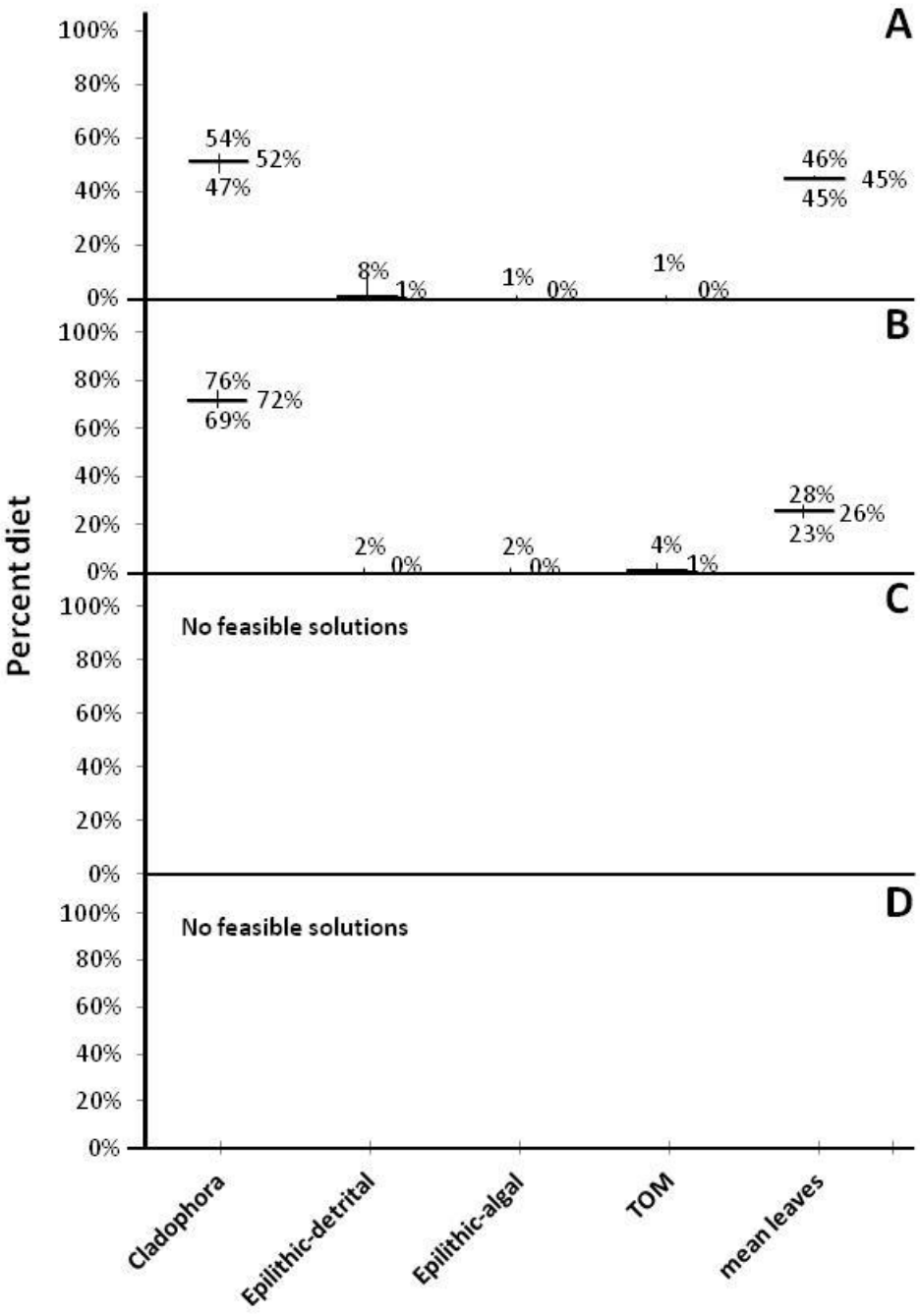

Figure 22: IsoSource results for *H. simulans* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

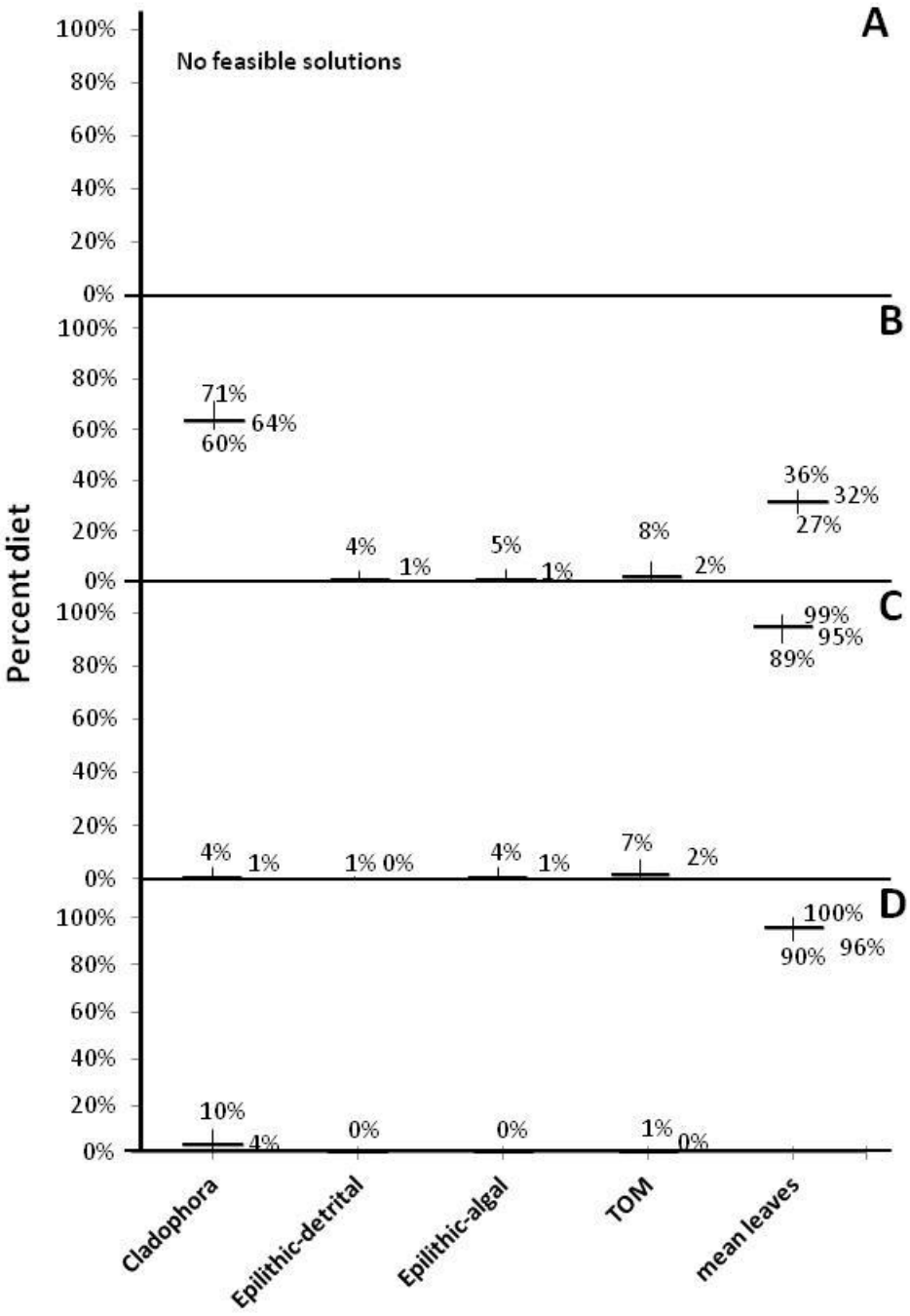

Figure 23: IsoSource results for *Isonychia* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

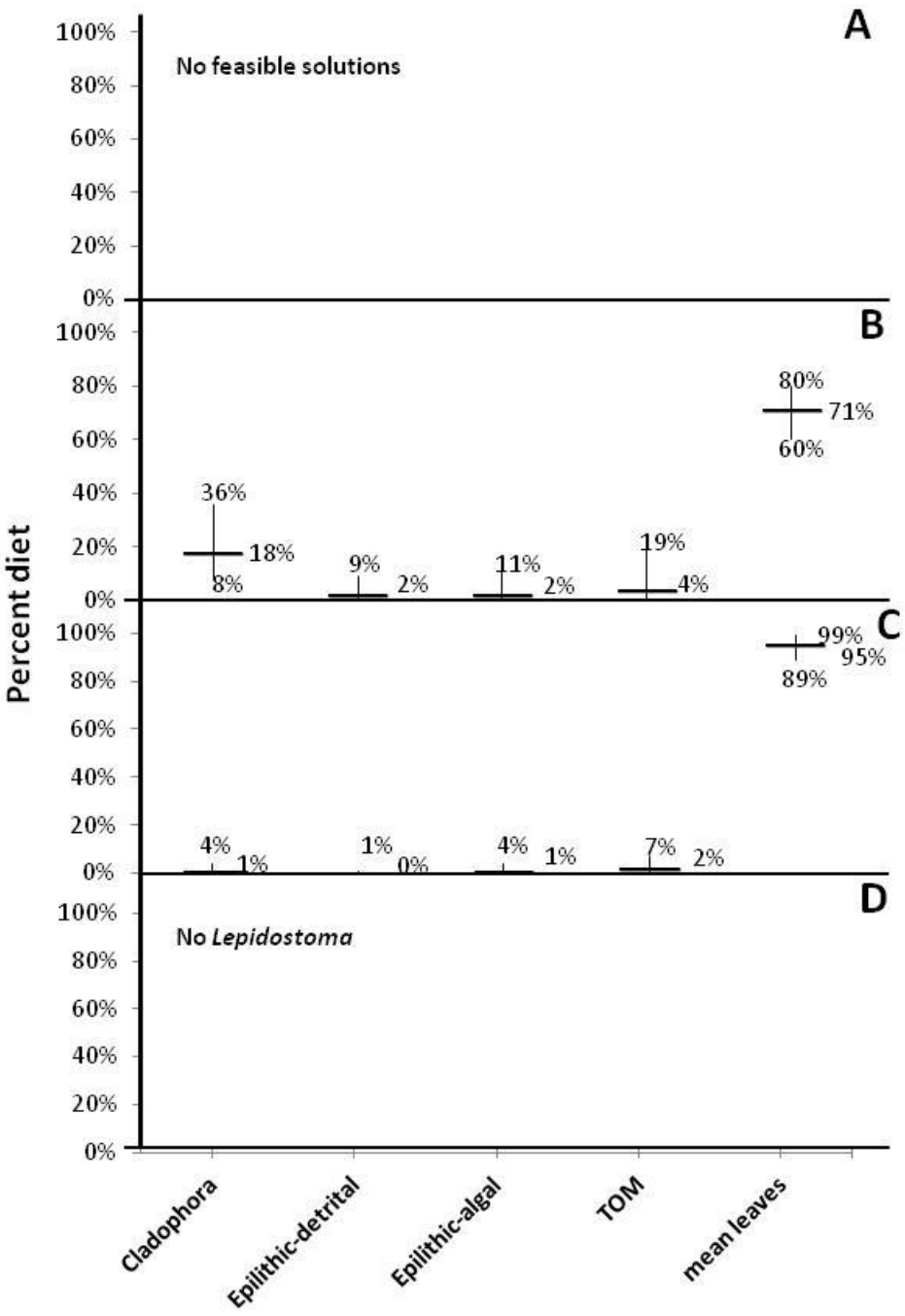

Figure 24: IsoSource results for *Lepidostoma* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

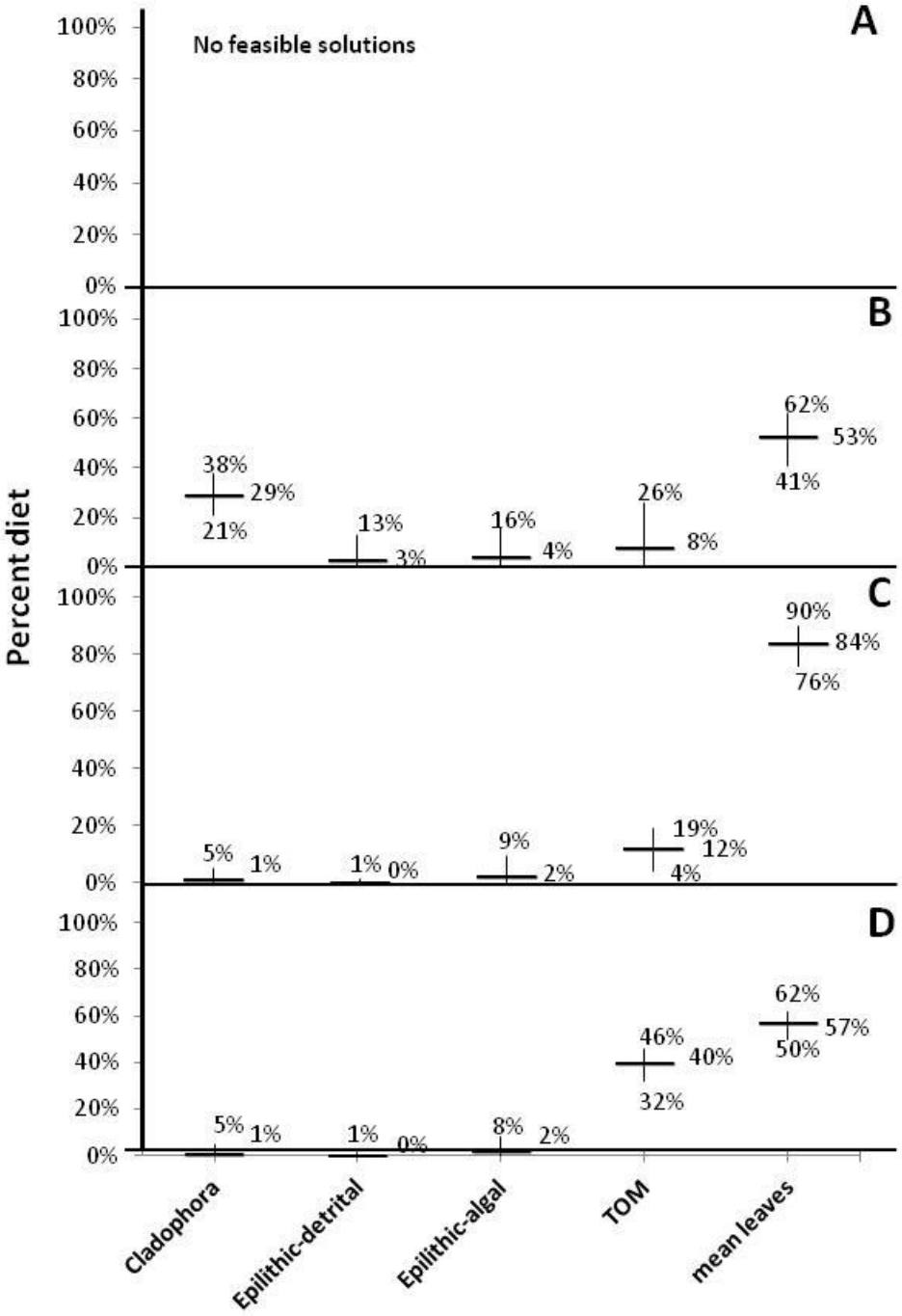

Figure 25: IsoSource results for *Maccaffertium mediopunctatum* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

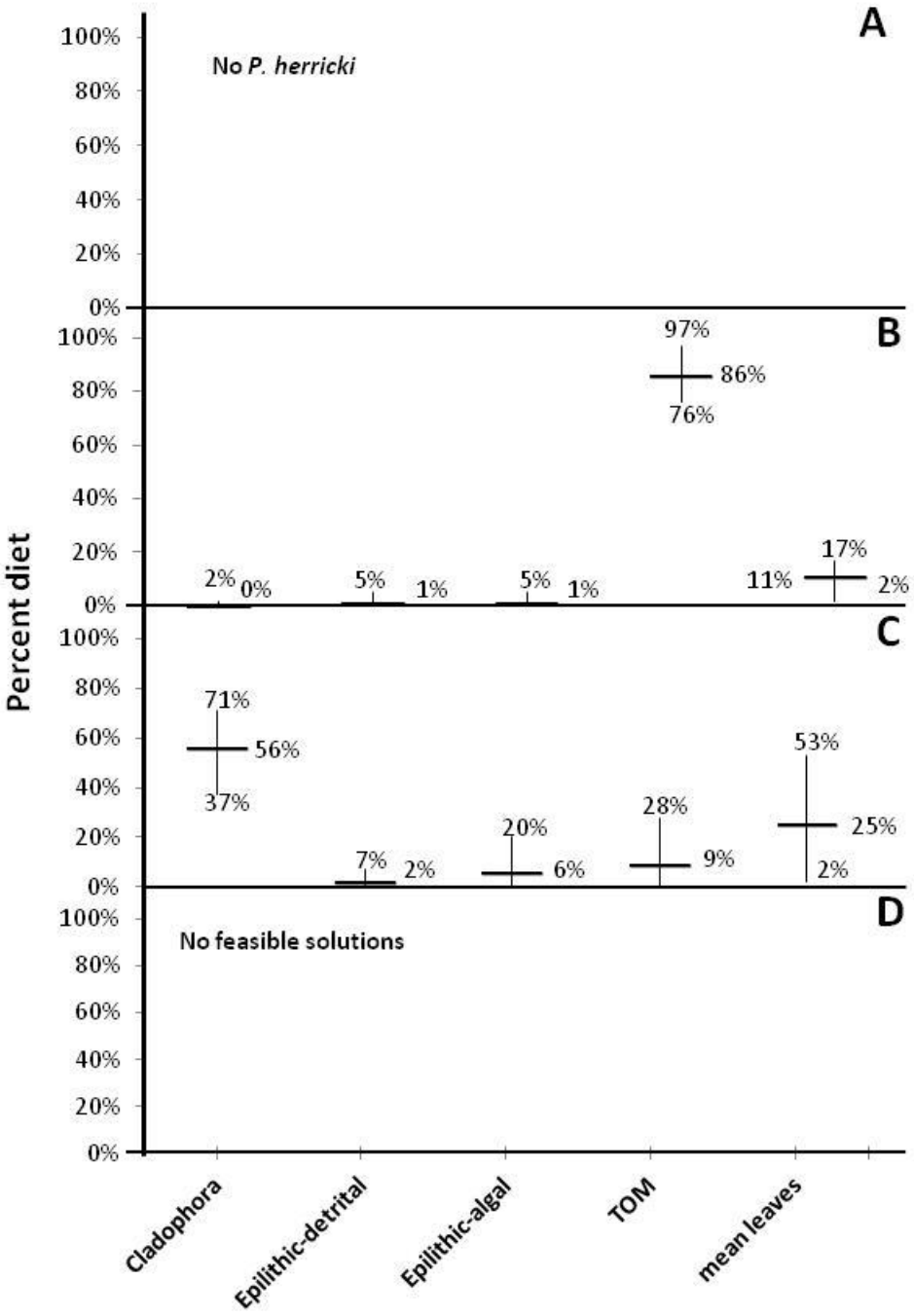

Figure 26: IsoSource results for *P. herricki* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

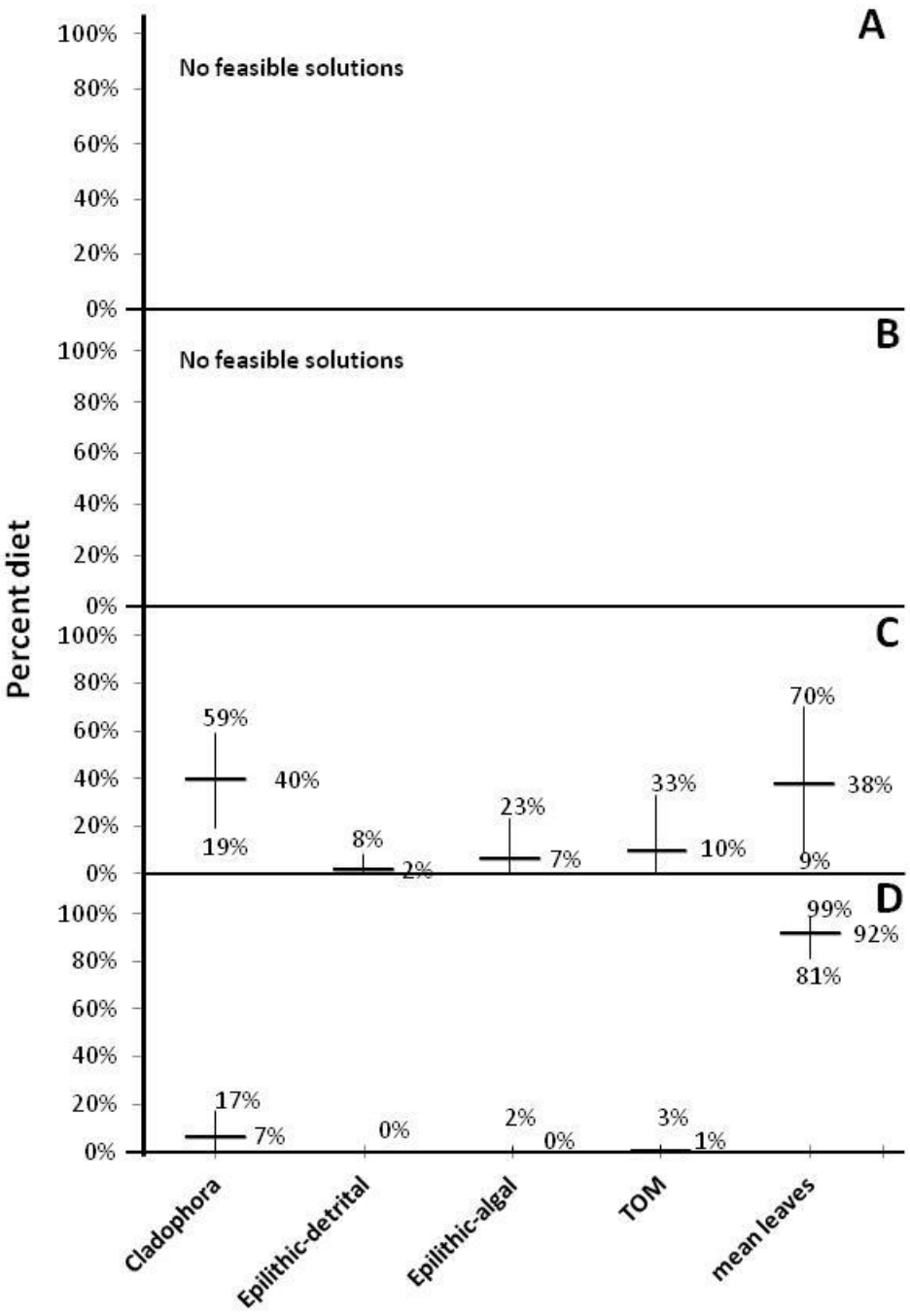

Figure 27: IsoSource results for *Pycnopsyche* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

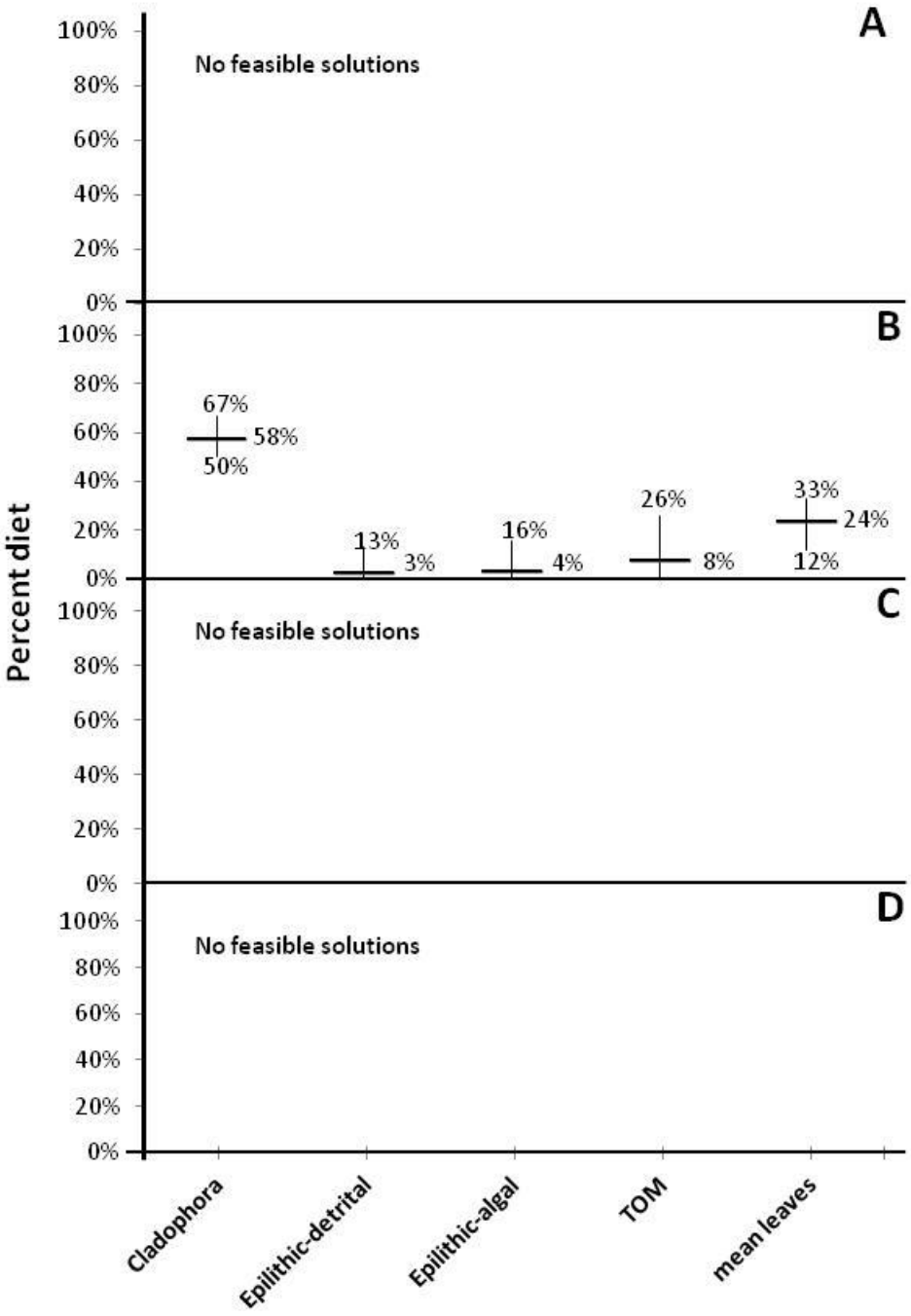

Figure 28: IsoSource results for *Simulium* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

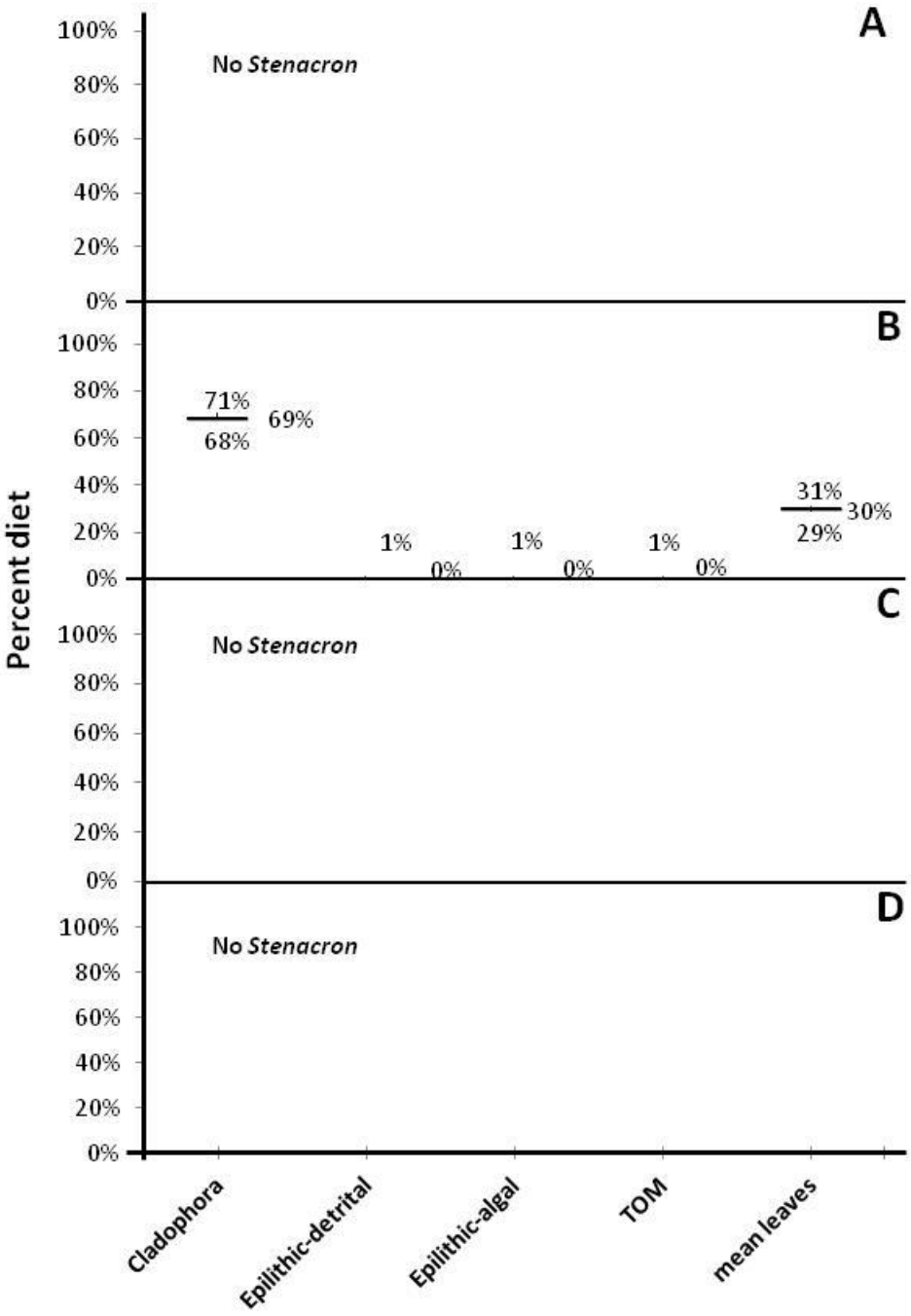

Figure 29: IsoSource results for *Stenacron* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

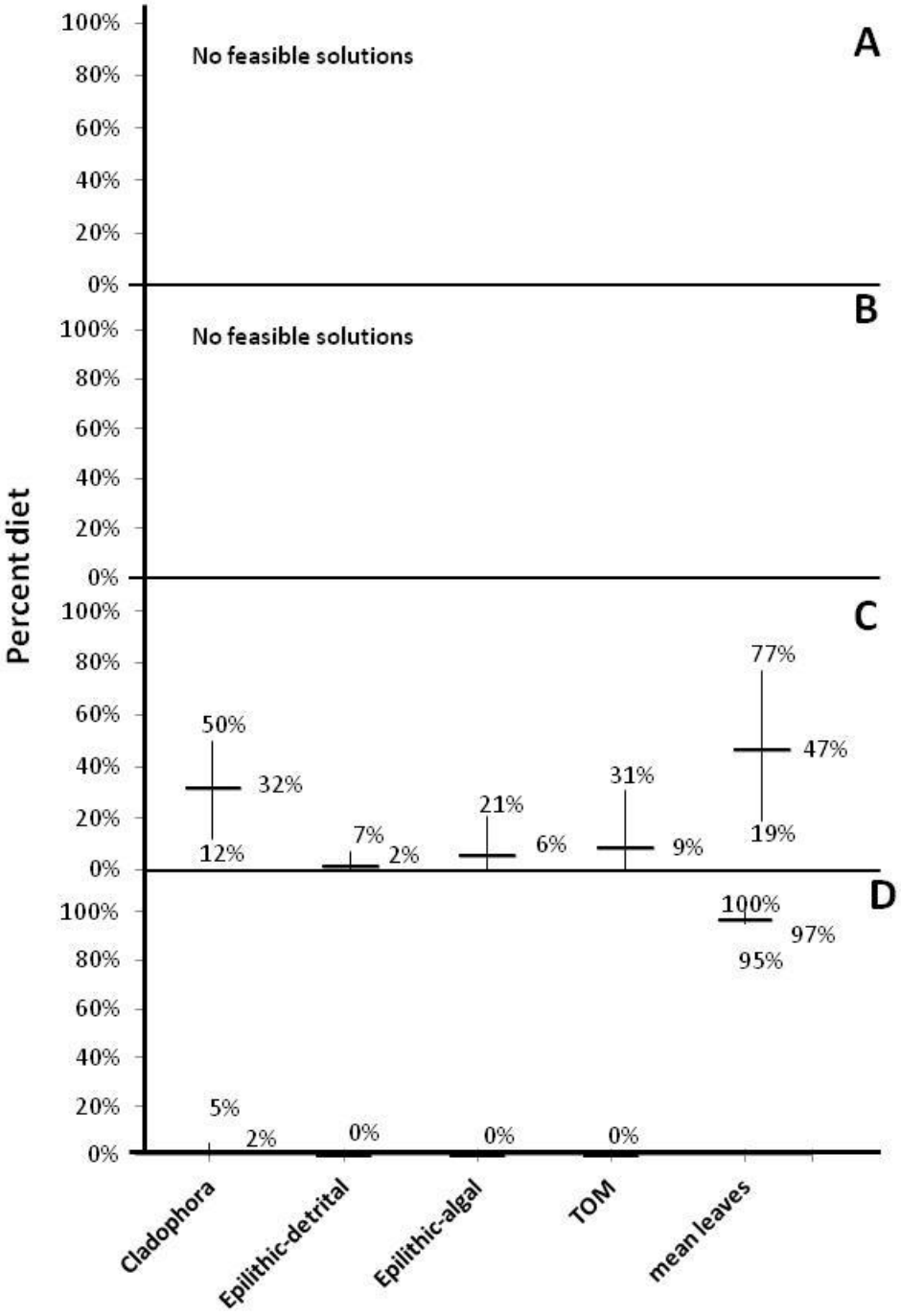

Figure 30: IsoSource results for *S. crenata* and five potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

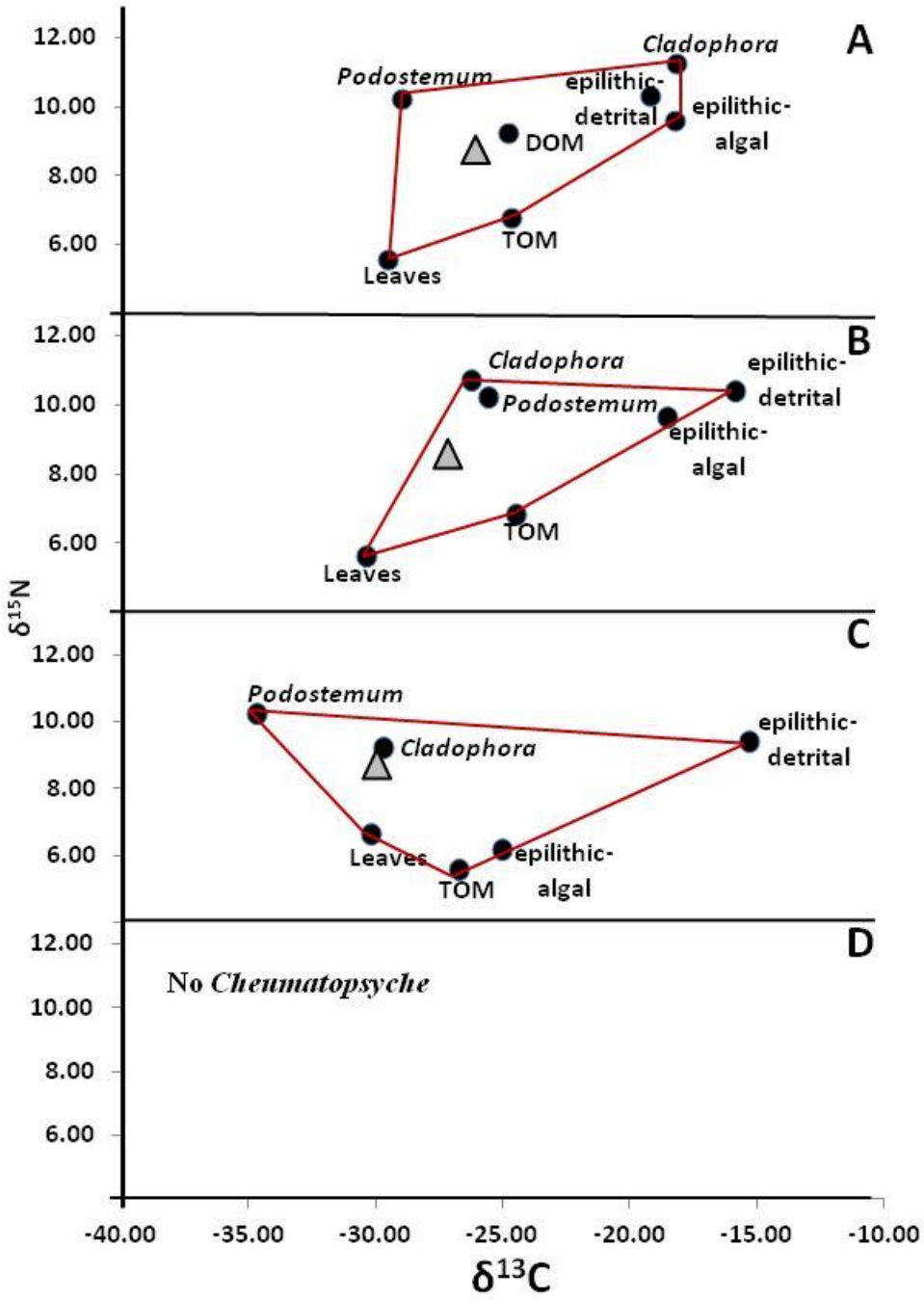

Figure 31: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Cheumatopsyche* and six or seven potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream.

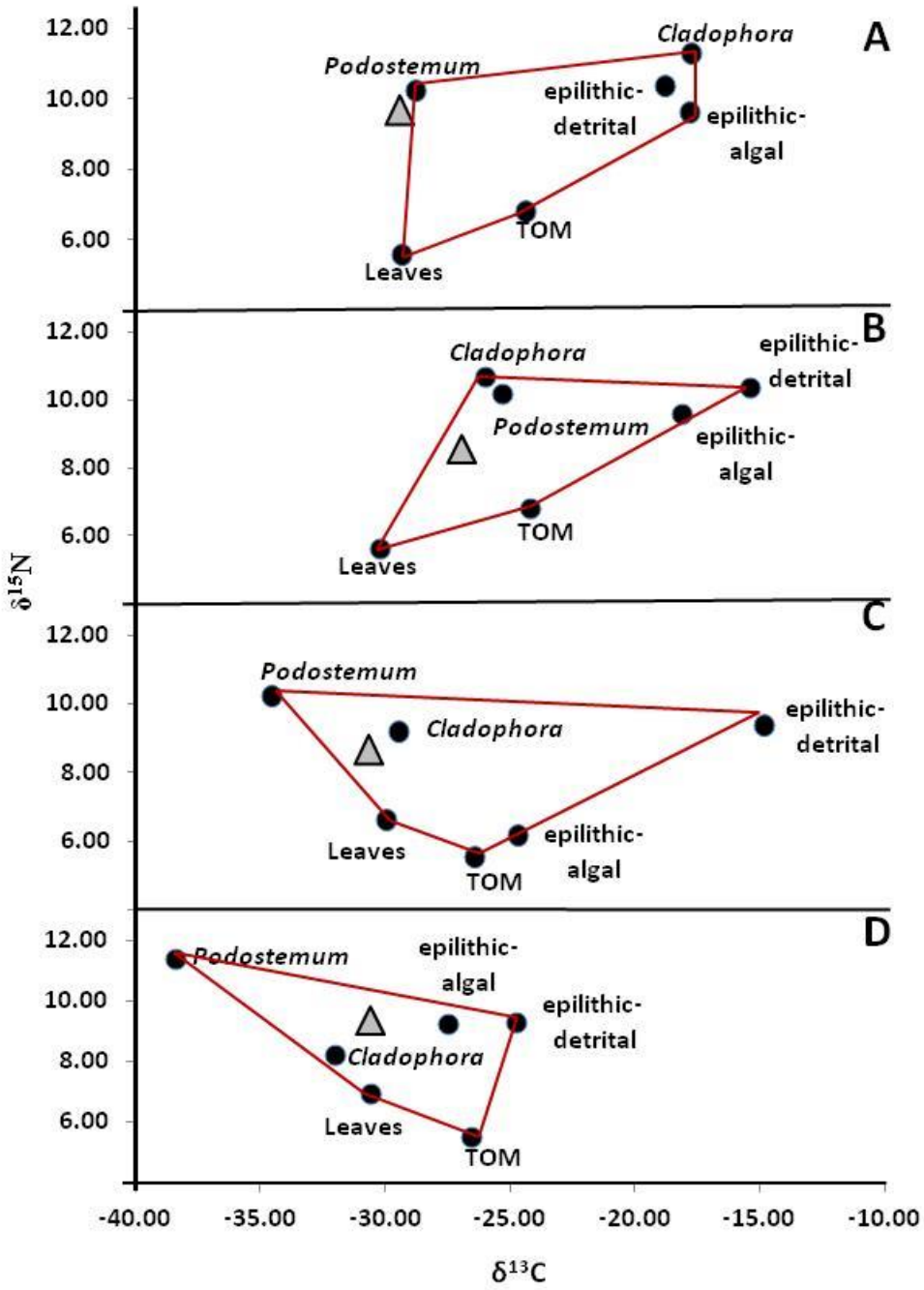

Figure 32: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *C. fluminea* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream.

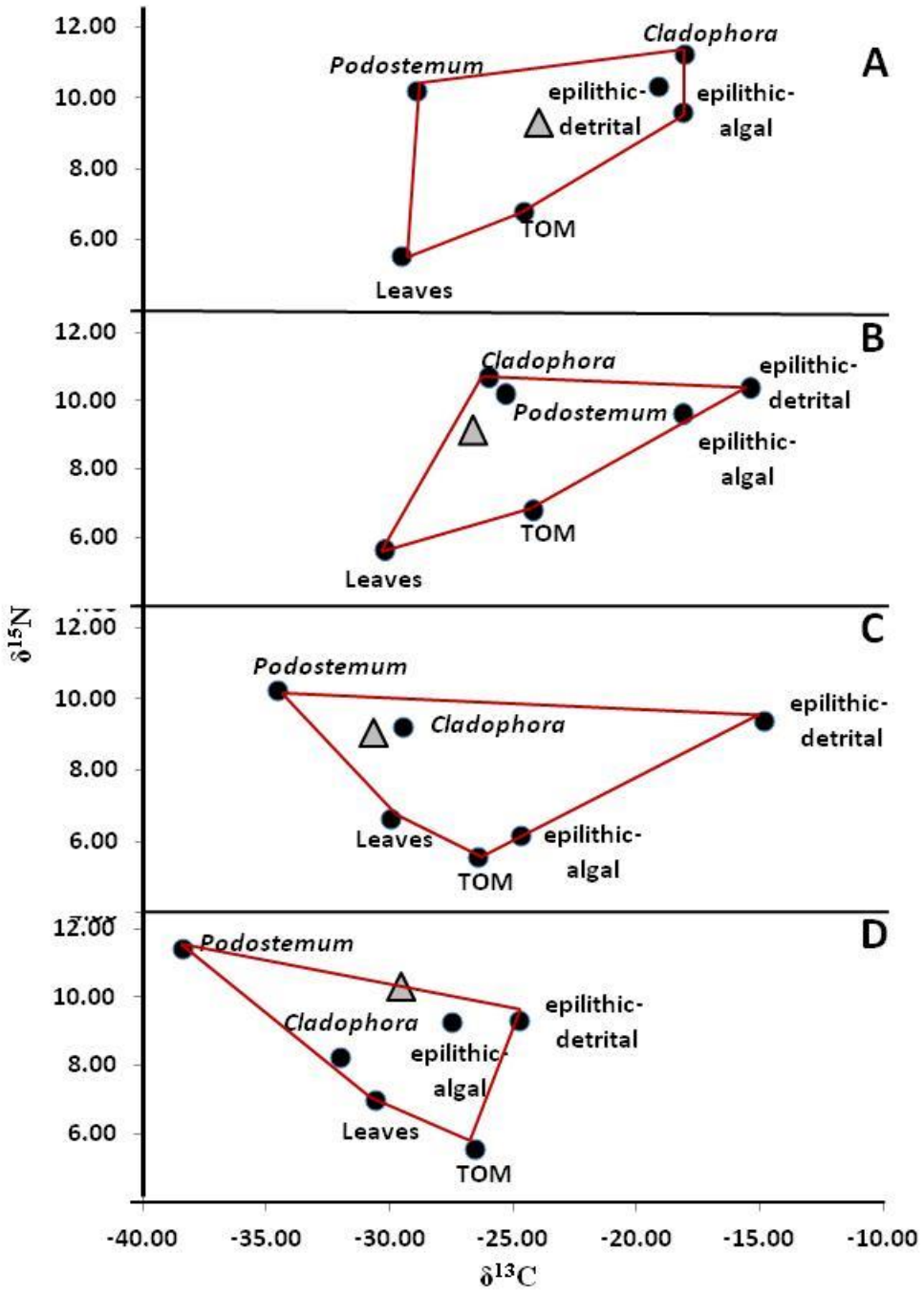

Figure 33: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *H. simulans* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream.

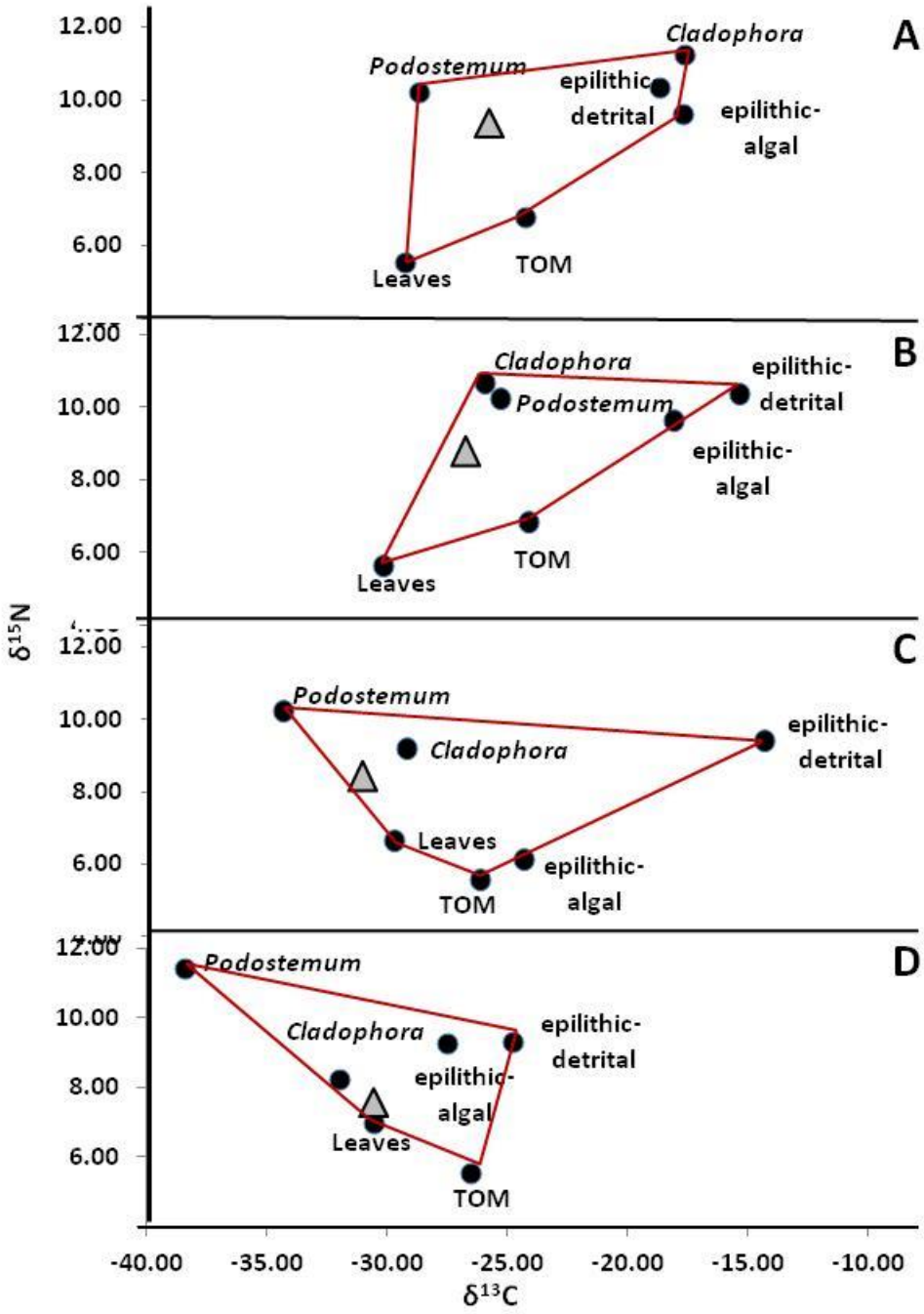

Figure 34: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Isonychia* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream.

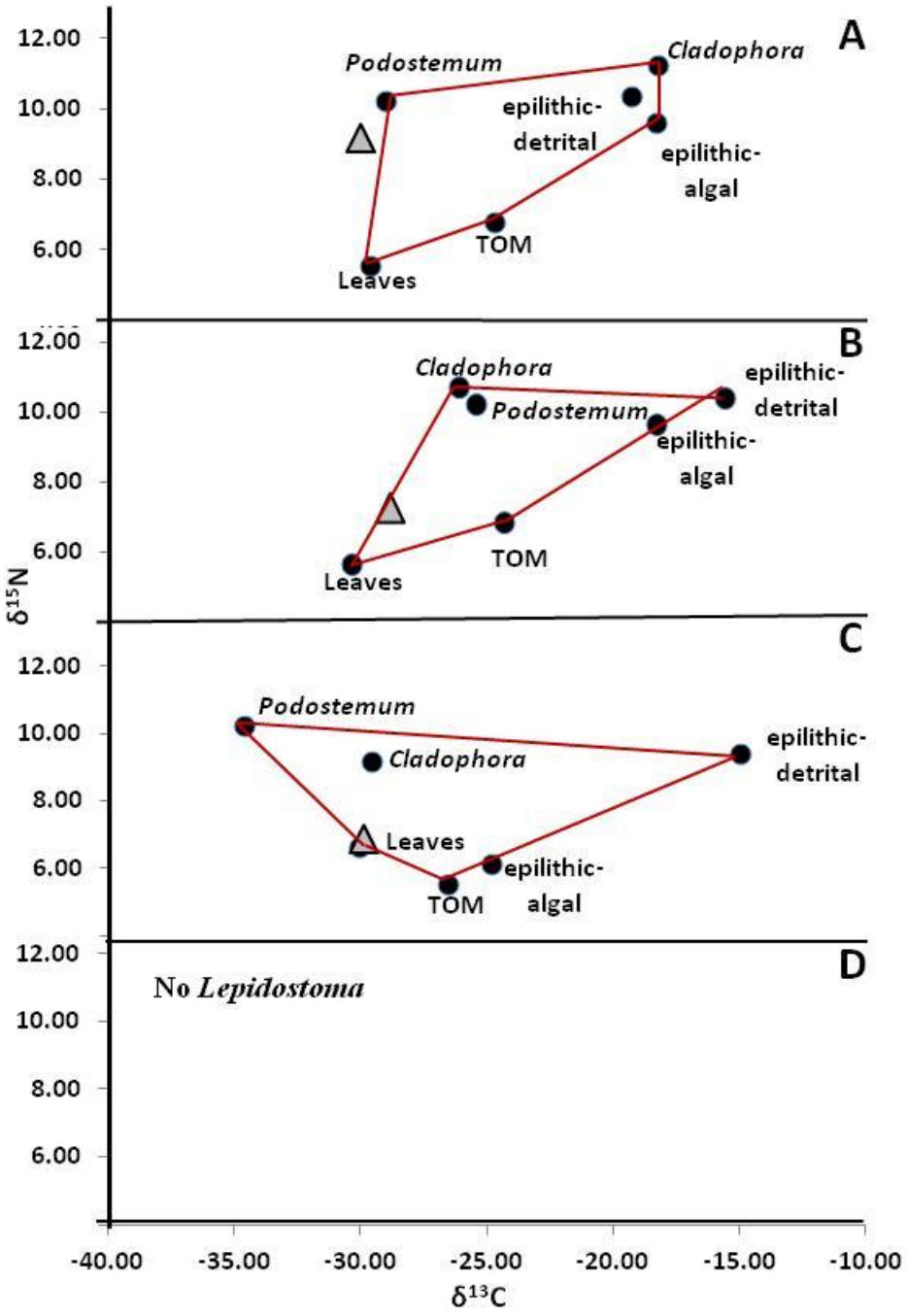

Figure 35: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Lepidostoma* and six potential food resources in four reaches along the upper Green River during summer 2012. Reaches A through D are in order from upstream to downstream.

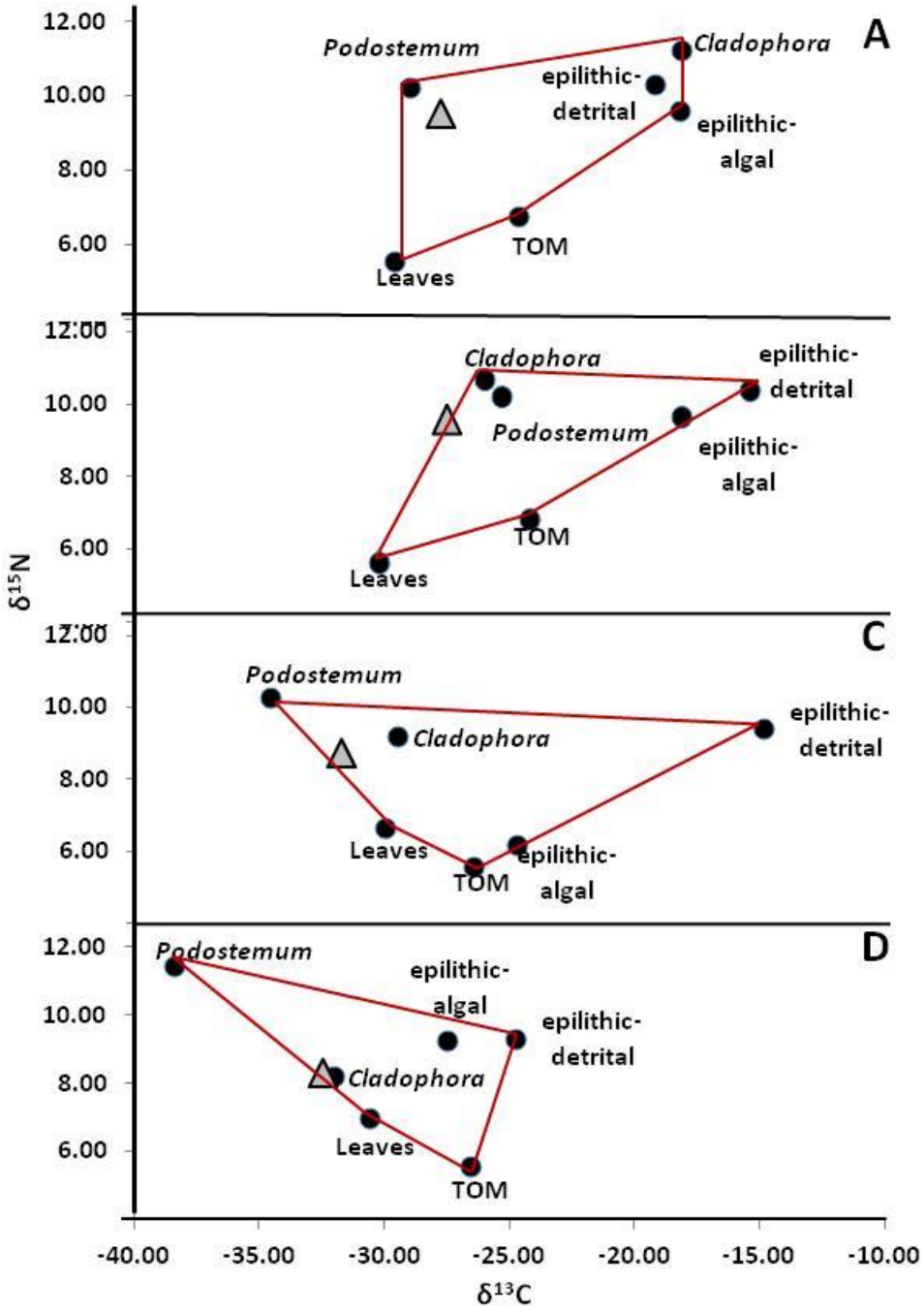

Figure 36: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *L. praerosa* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

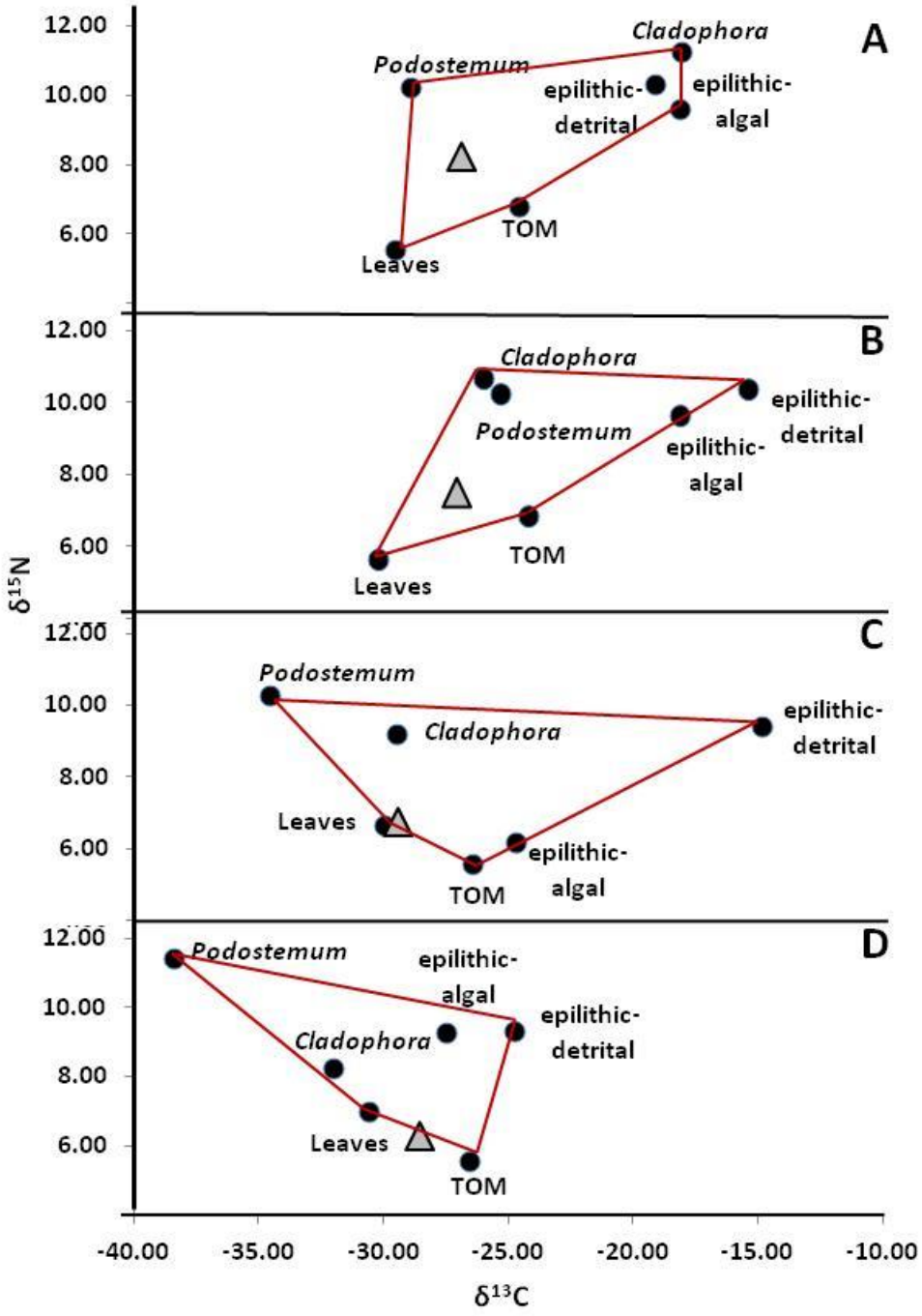

Figure 37: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Maccaffertium mediopunctatum* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

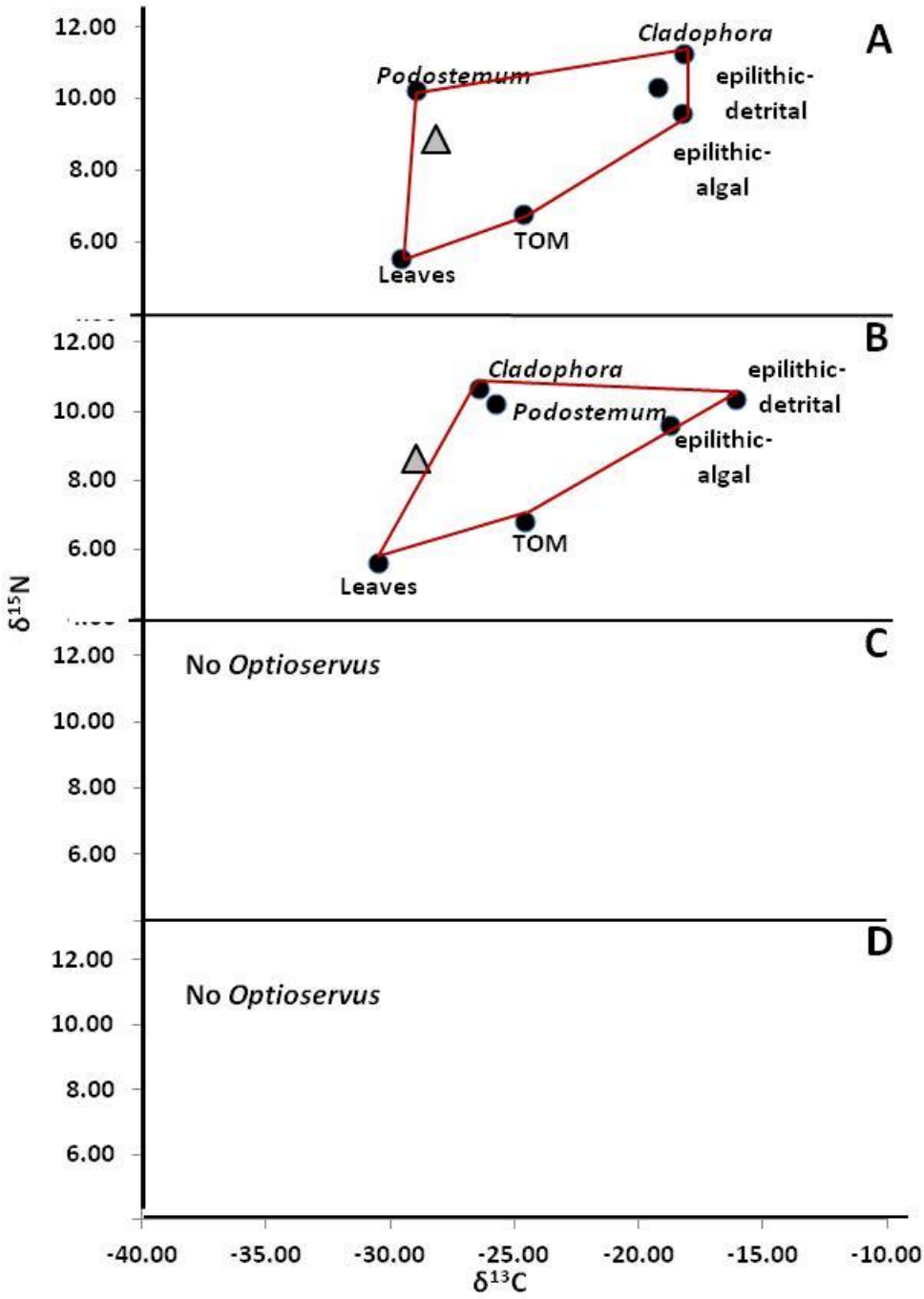

Figure 38: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Optioservus* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

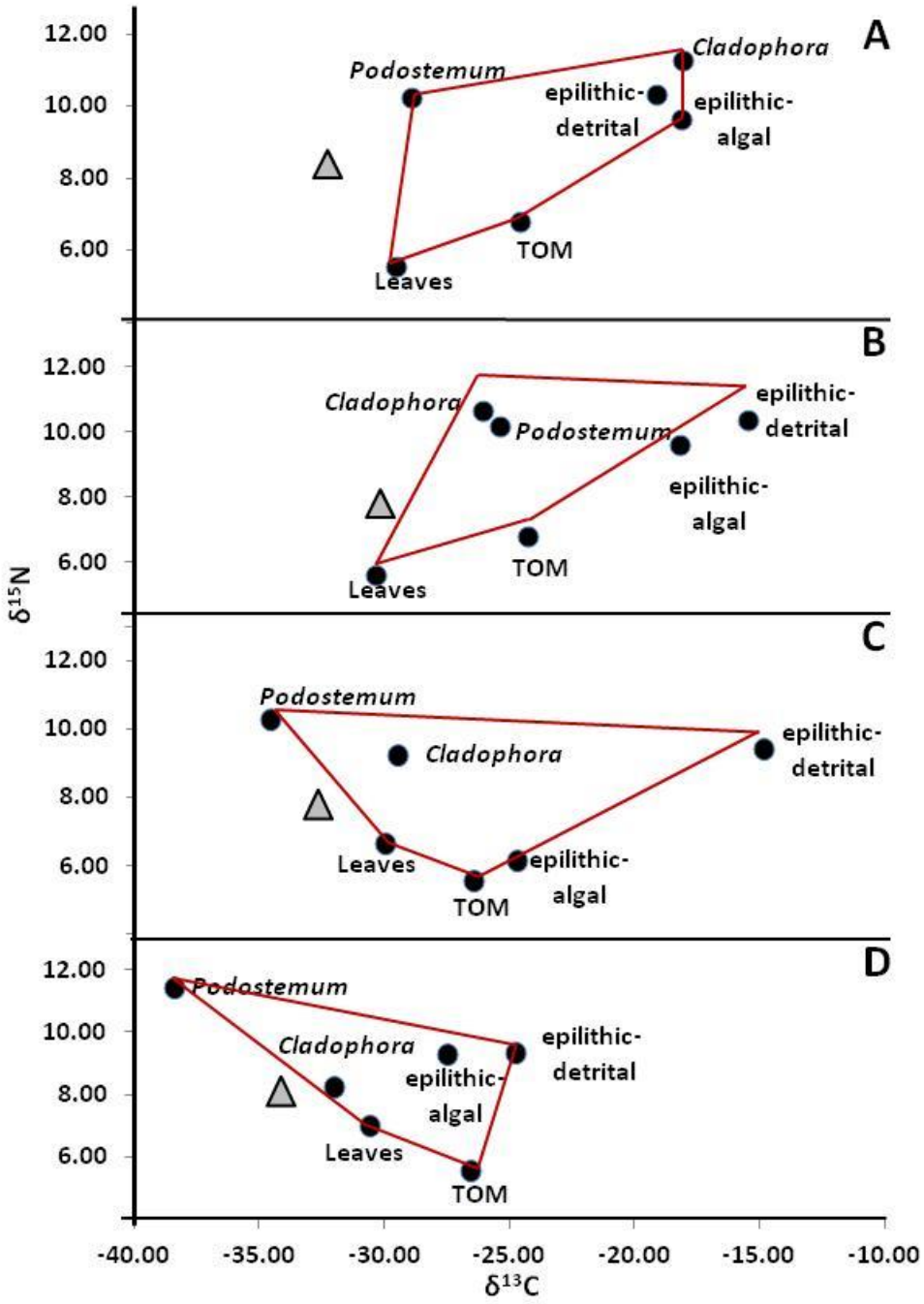

Figure 39: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *P. dorsata* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

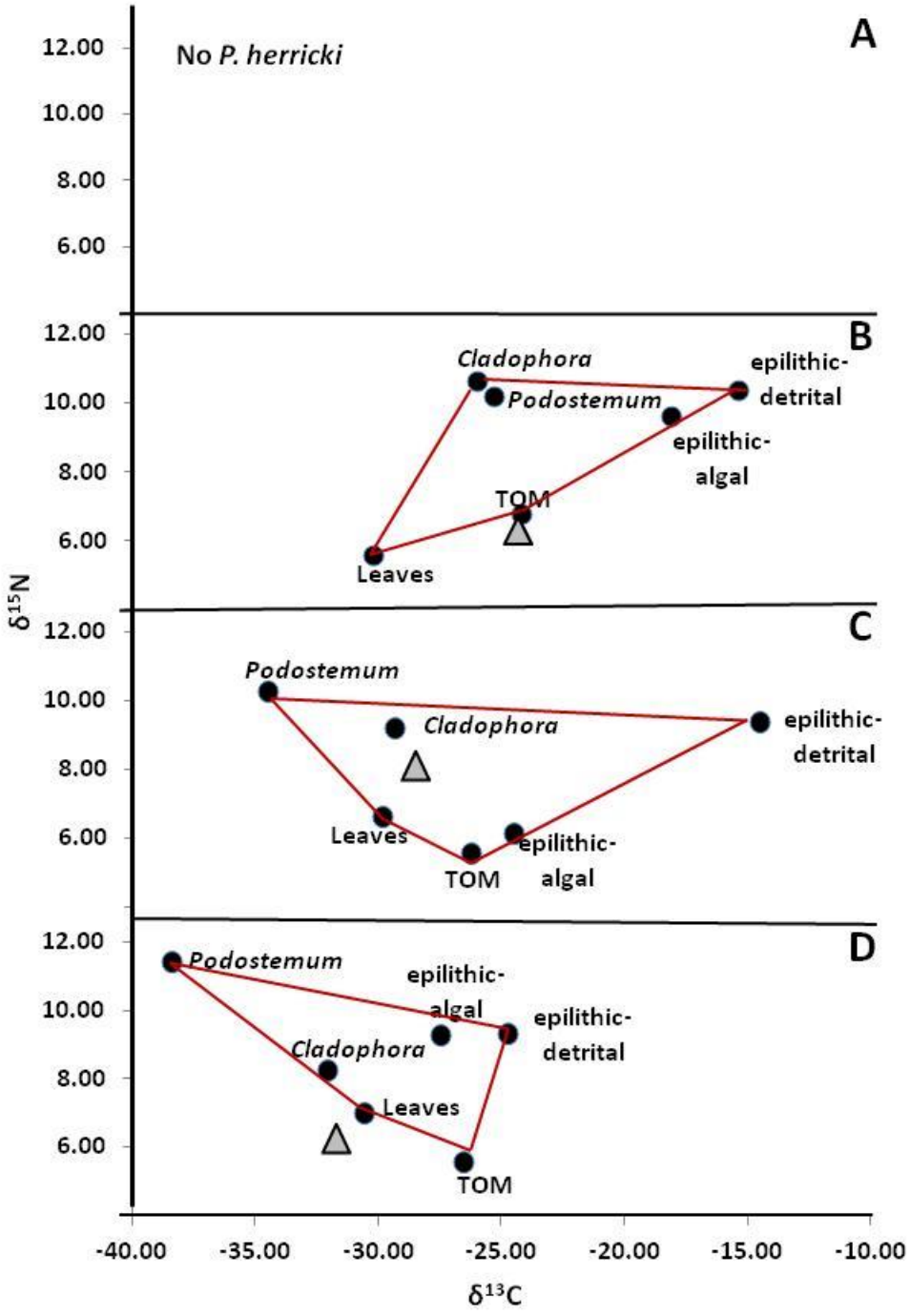

Figure 40: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *P. herricki* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

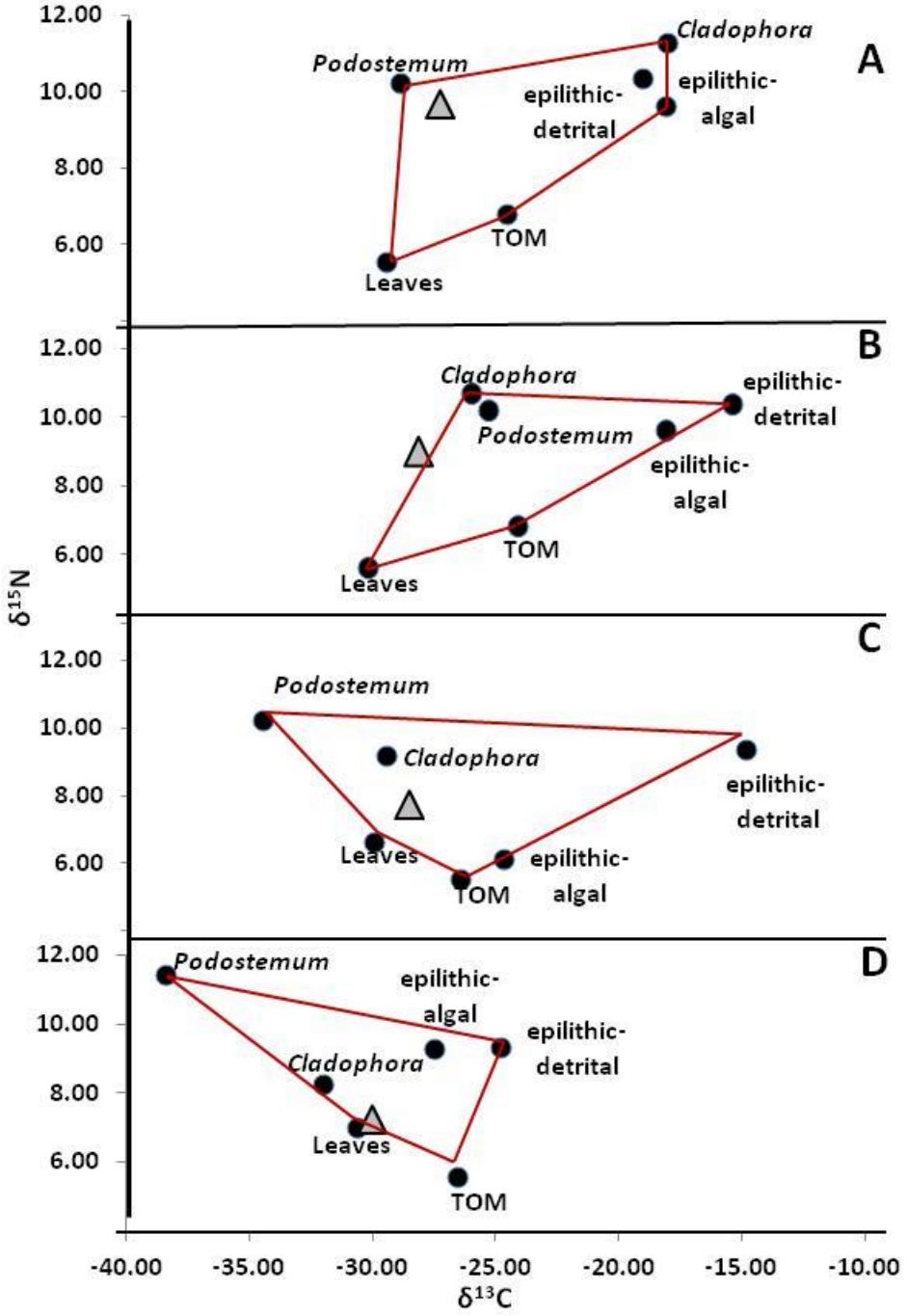

Figure 41: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Pycnopsyche* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

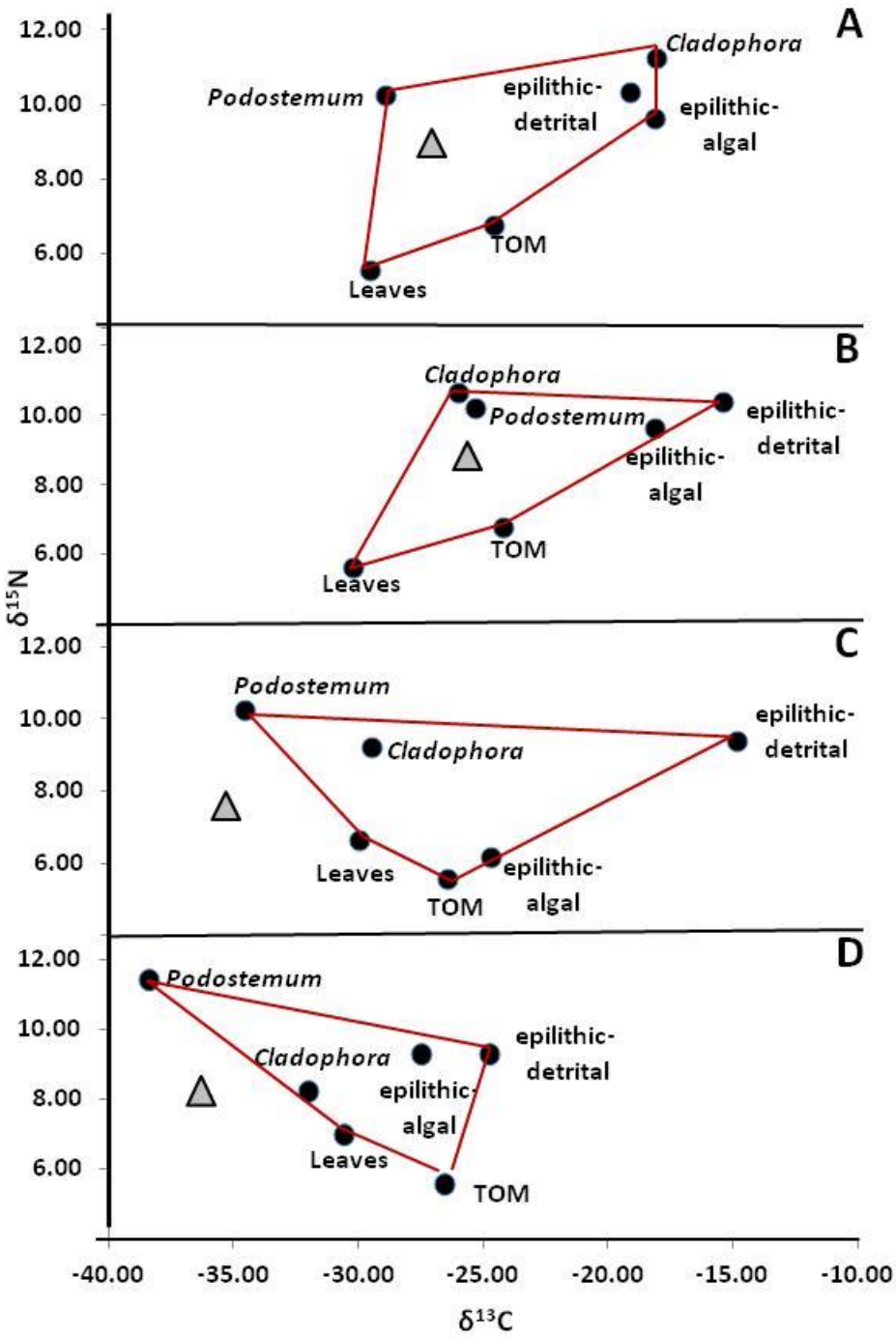

Figure 42: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Simulium* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

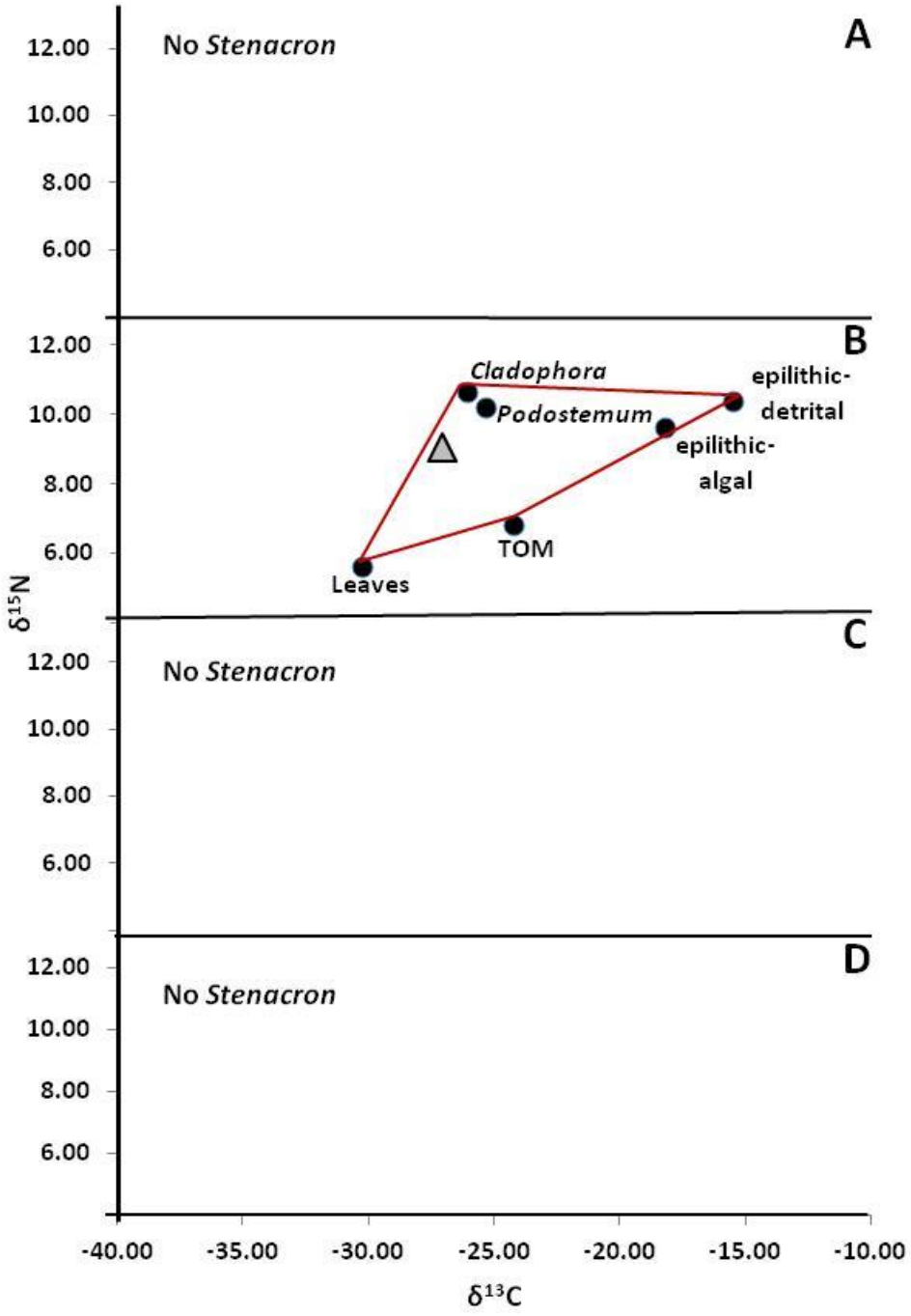

Figure 43: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Stenacron* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

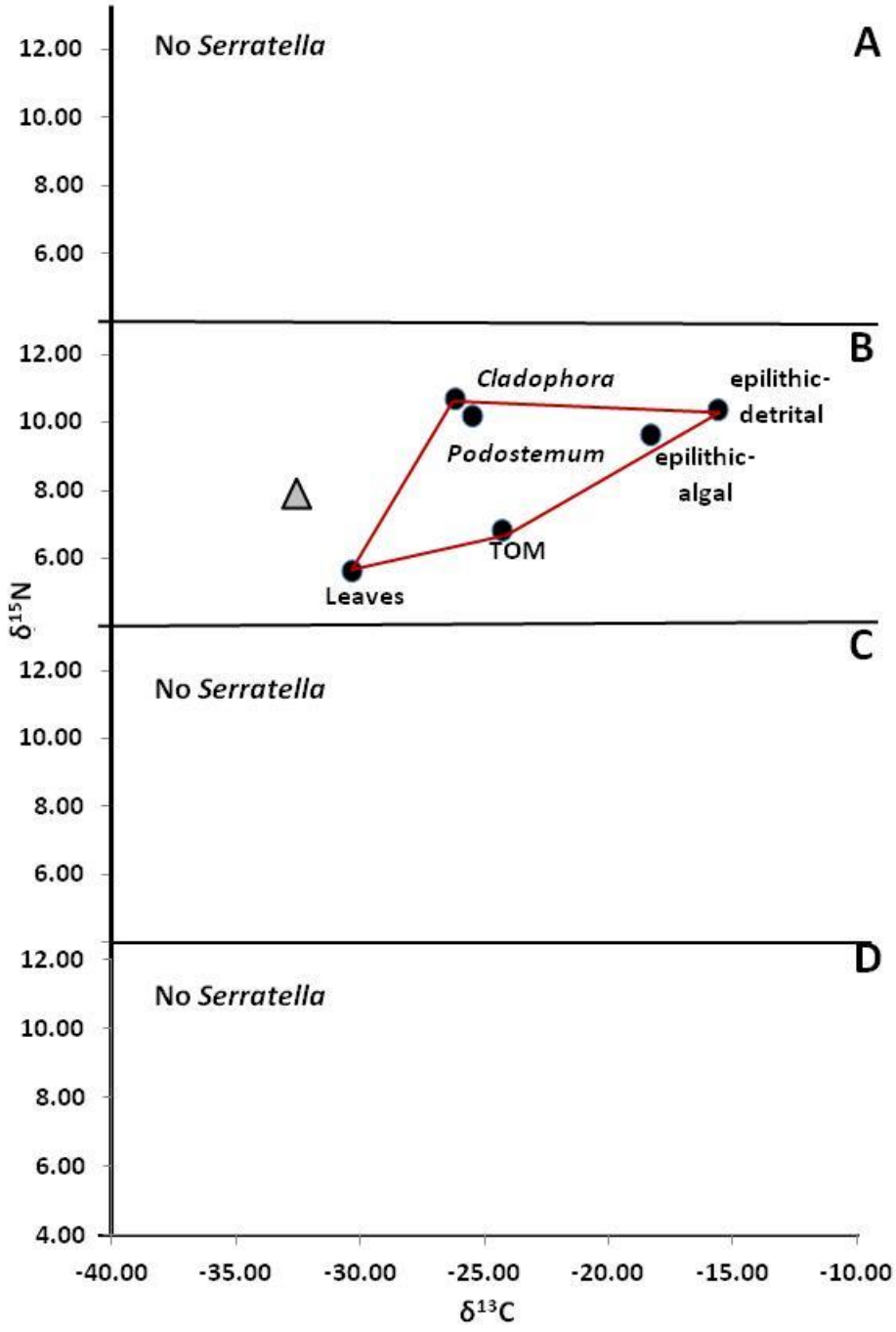

Figure 44: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Serratella* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

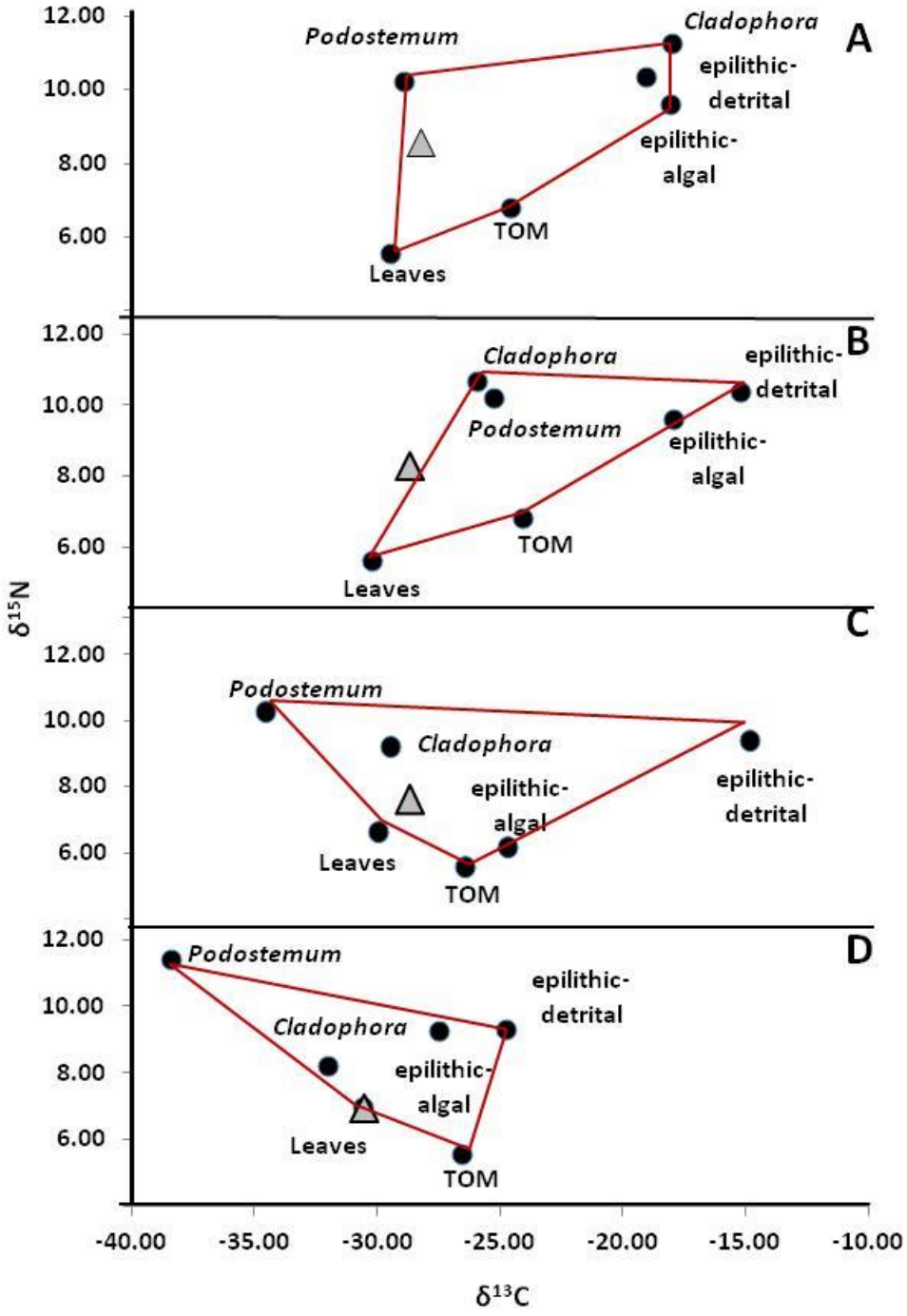

Figure 45: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *S. crenata* and six potential food sources during summer 2012. Sites A through D are the four study reaches along the upper Green River in Kentucky from upstream to downstream.

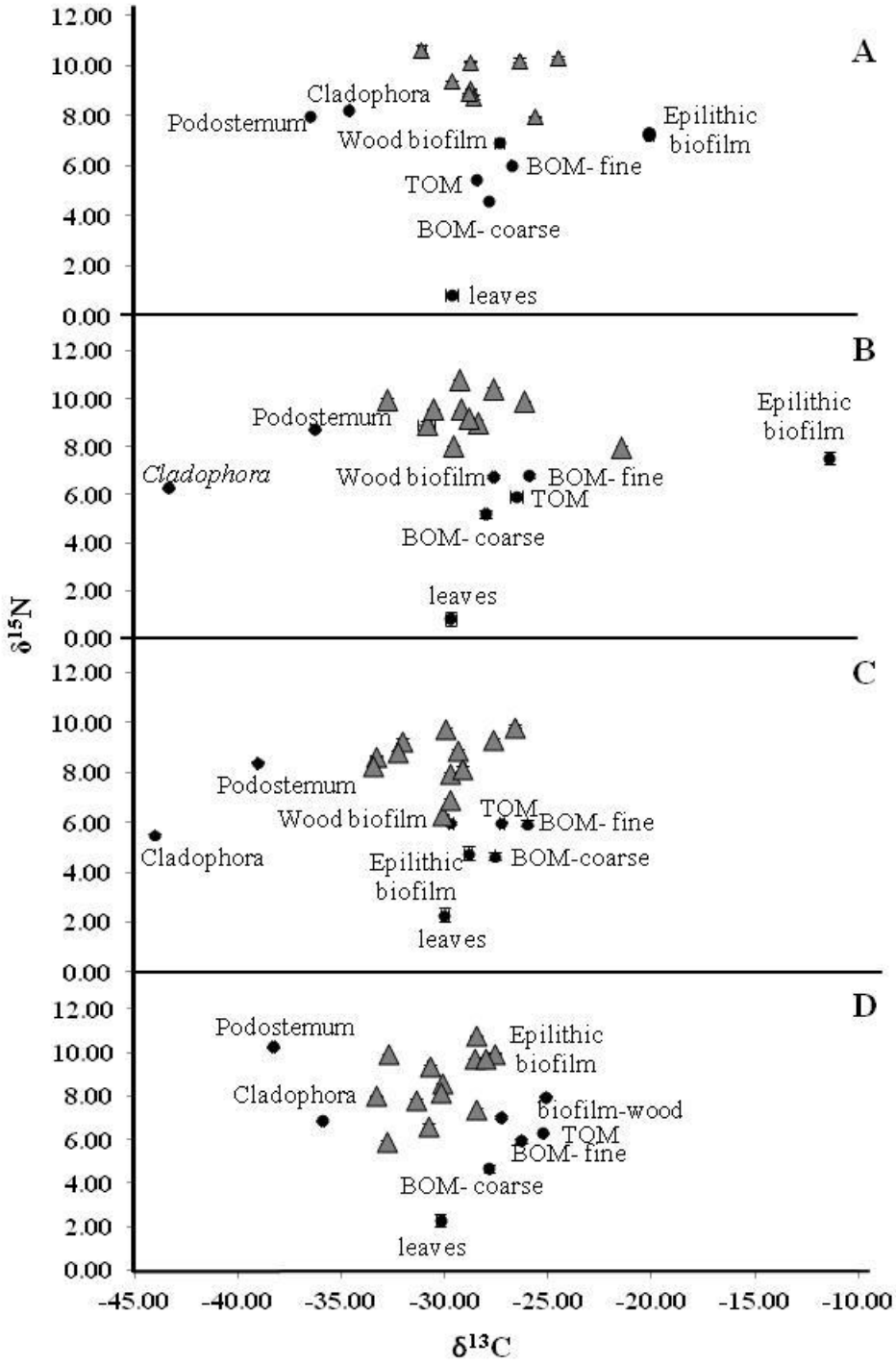

Figure 46: Stable isotope biplot of primary consumers and potential food resources at four reaches along the upper Green River during autumn 2013. Potential resources are depicted as circles with ±1SE. Primary consumer taxa are depicted as triangles and represent one sample per taxa. Reaches A through D are in order from upstream to downstream.

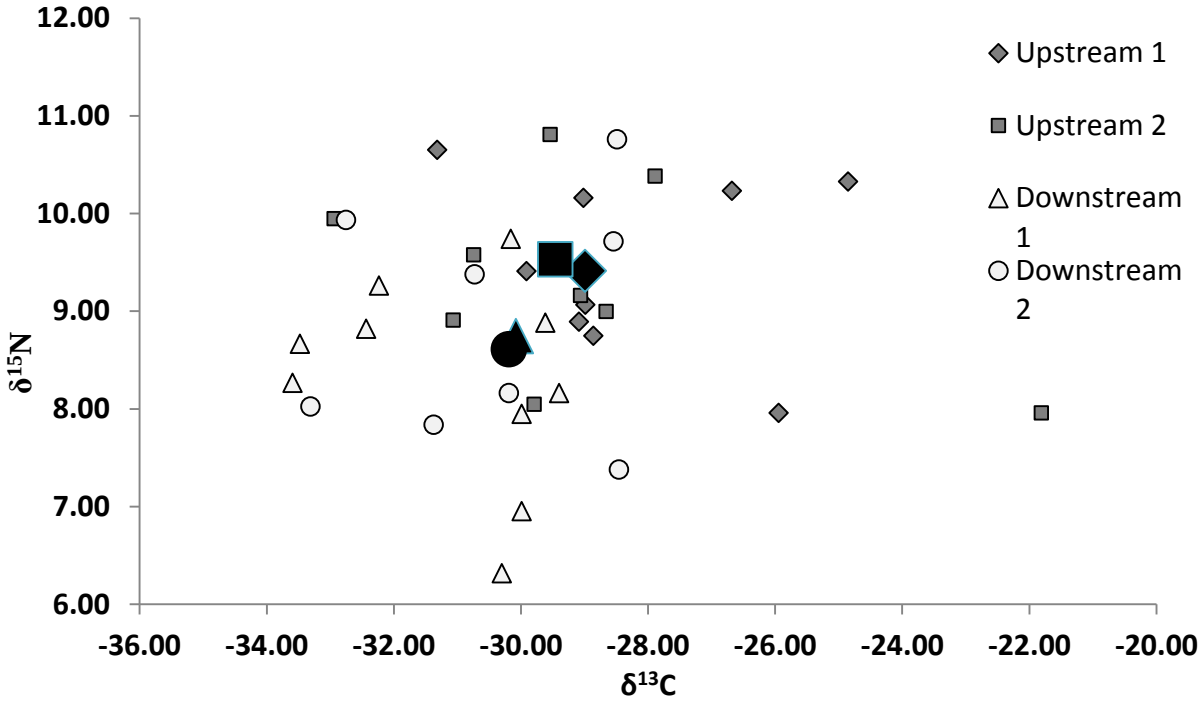

Figure 47: Stable isotope biplot of primary consumers at four study reaches along the Green River during autumn 2013. Larger shapes represent median consumer stable isotope values at each reach. Reaches A through D are in order from upstream to downstream.

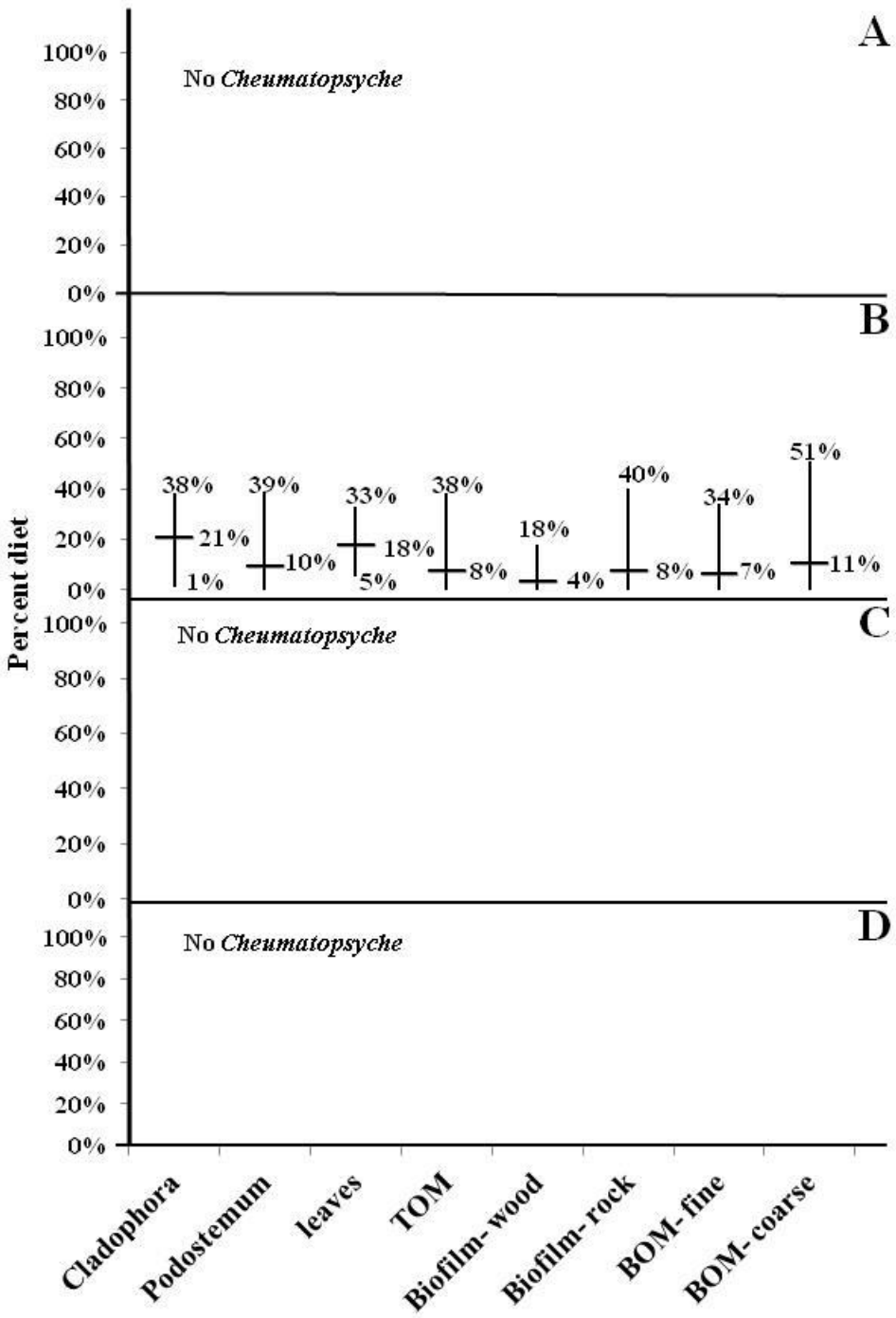

Figure 48: IsoSource results for *Cheumatopsyche* and eight potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

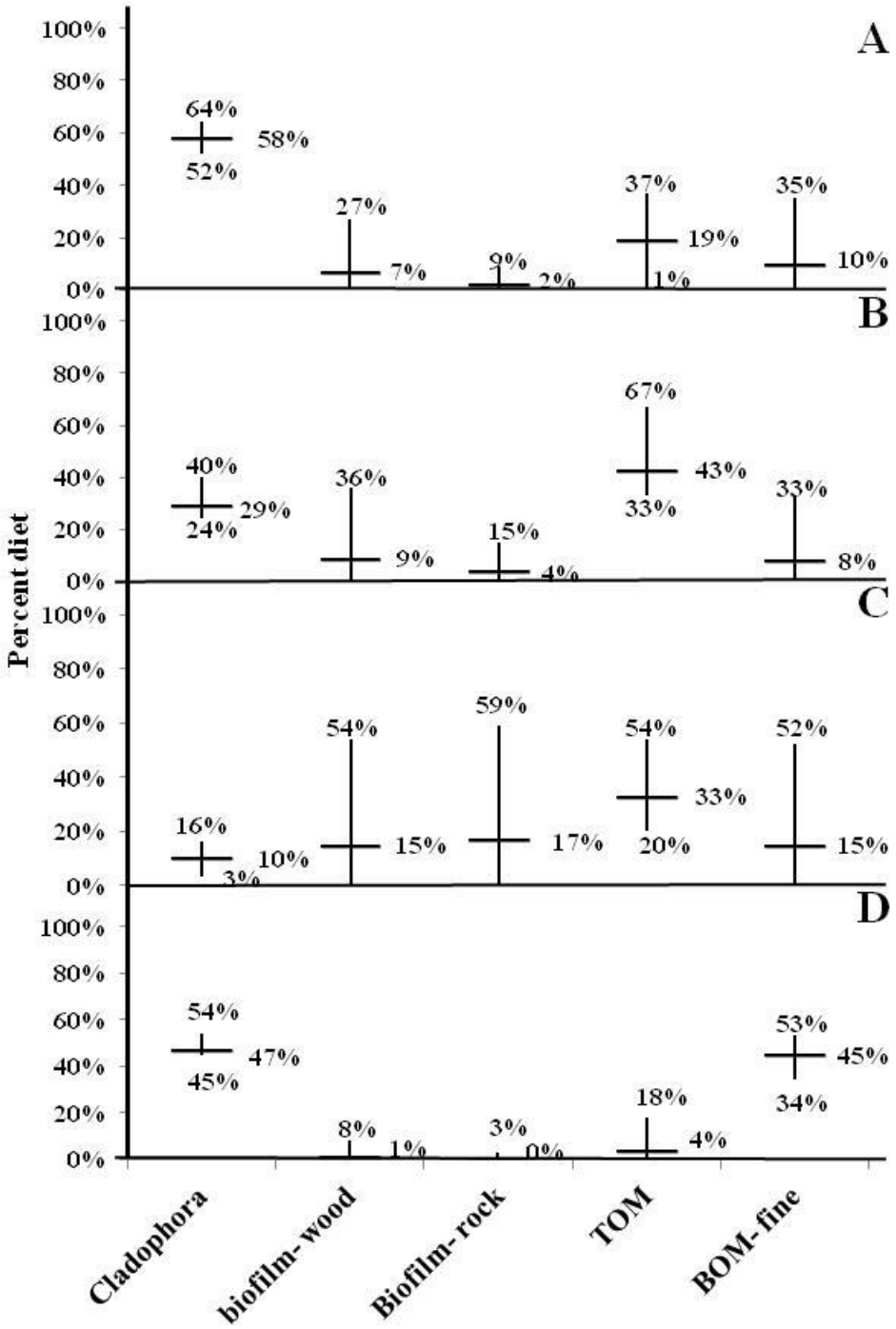

Figure 49: IsoSource results for *C. fluminea* and five potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

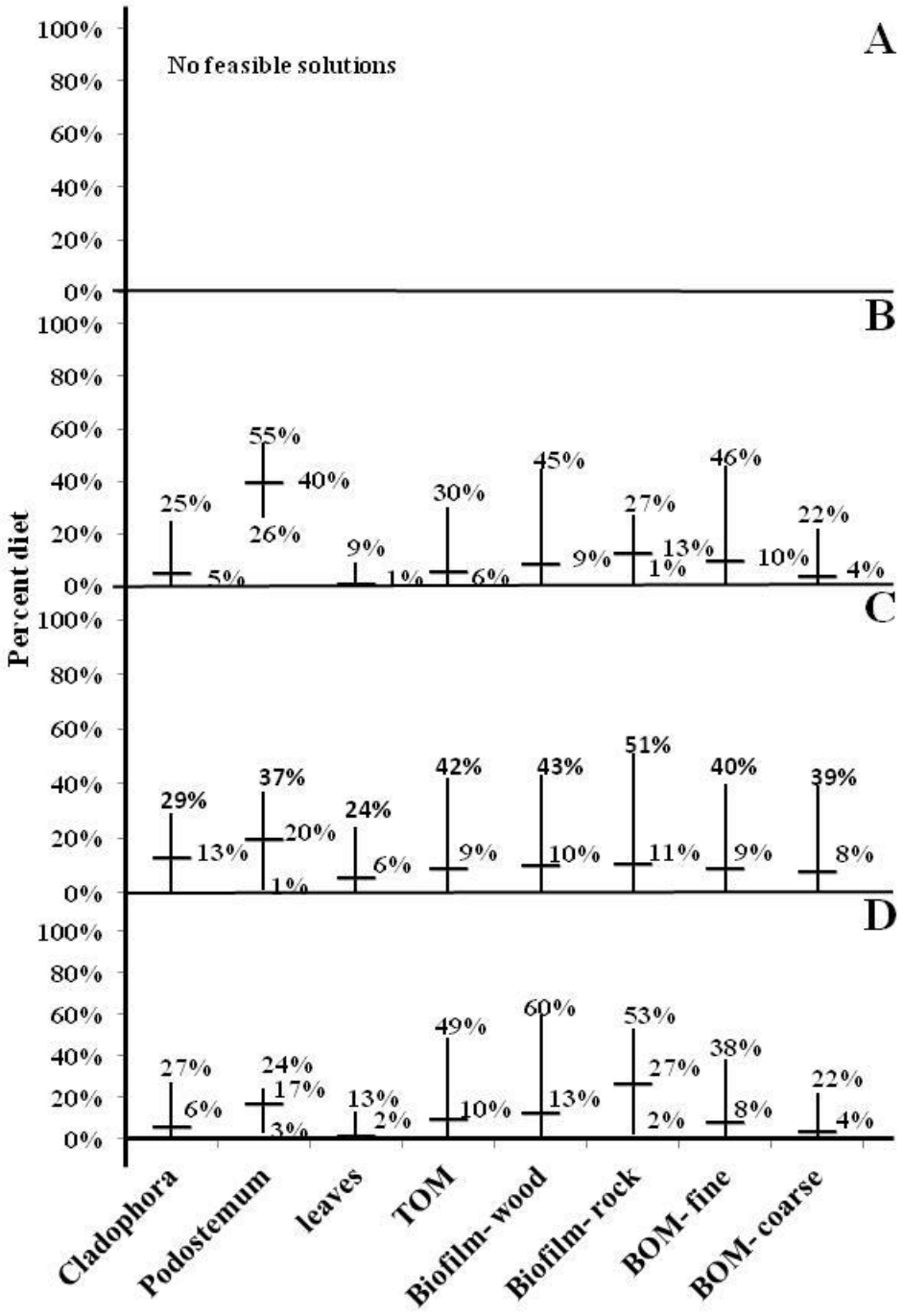

Figure 50: IsoSource results for *H. simulans* and eight potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

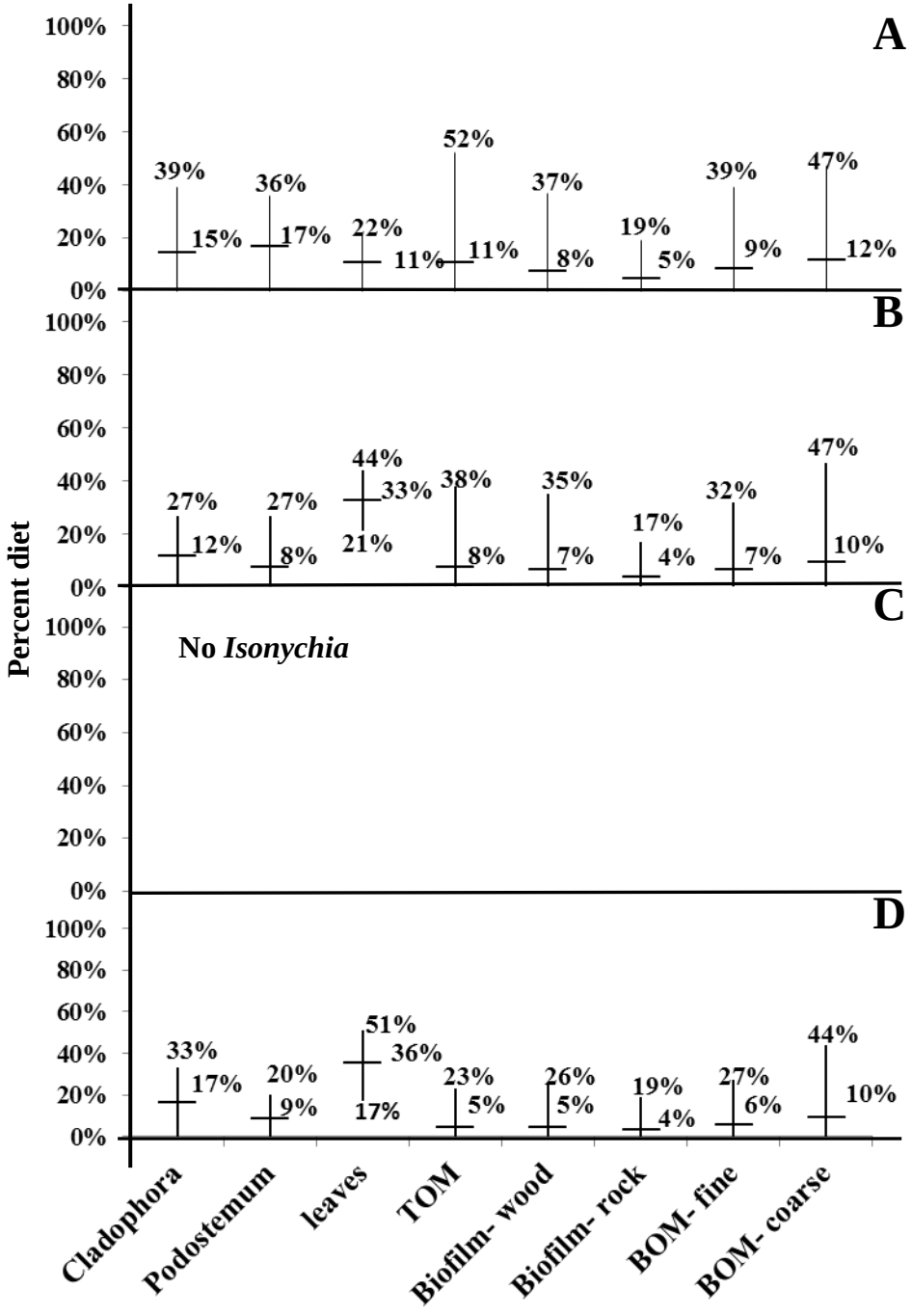

Figure 51: IsoSource results for *Isonychia* and eight potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

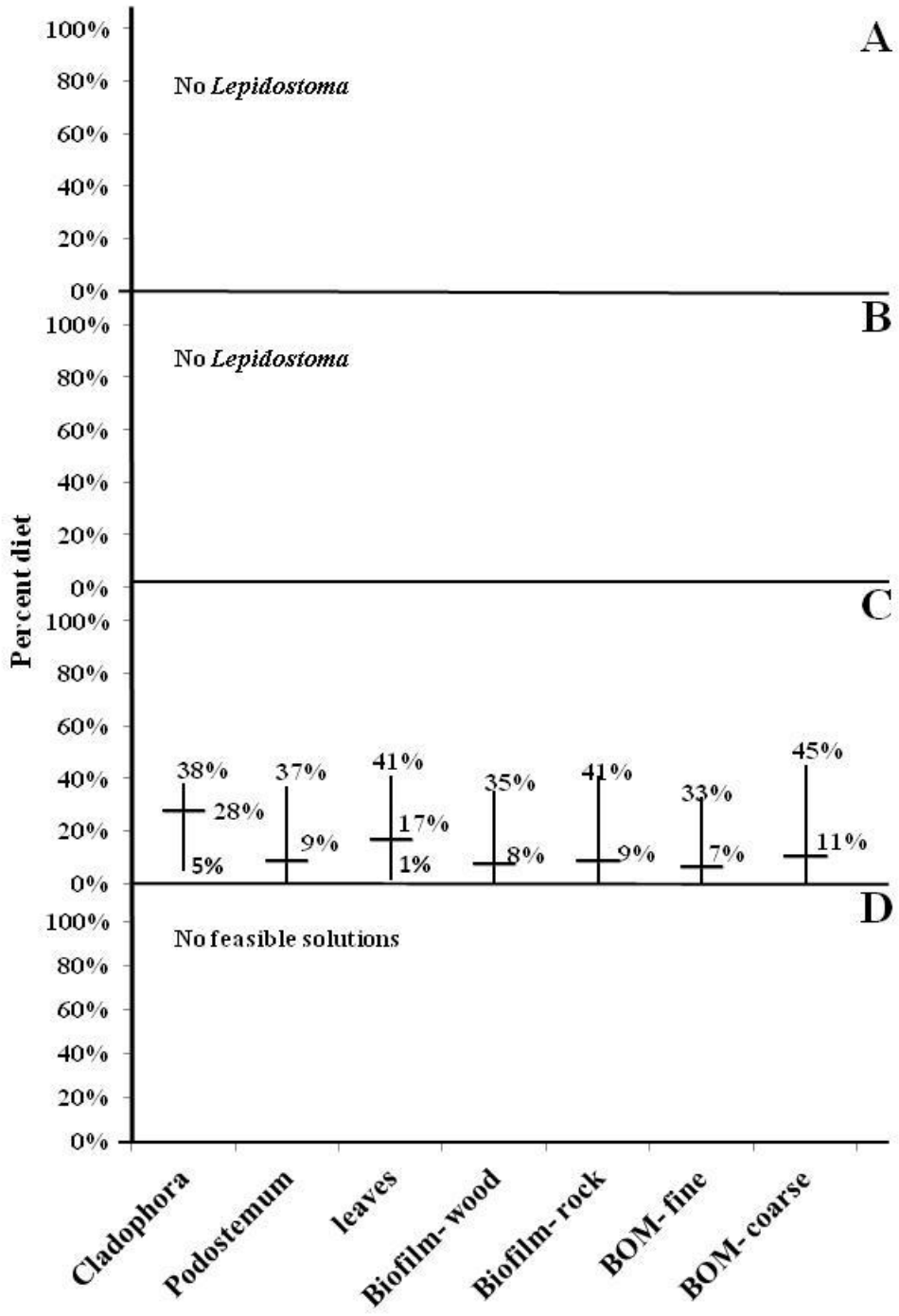

Figure 52: IsoSource results for *Lepidostoma* and seven potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

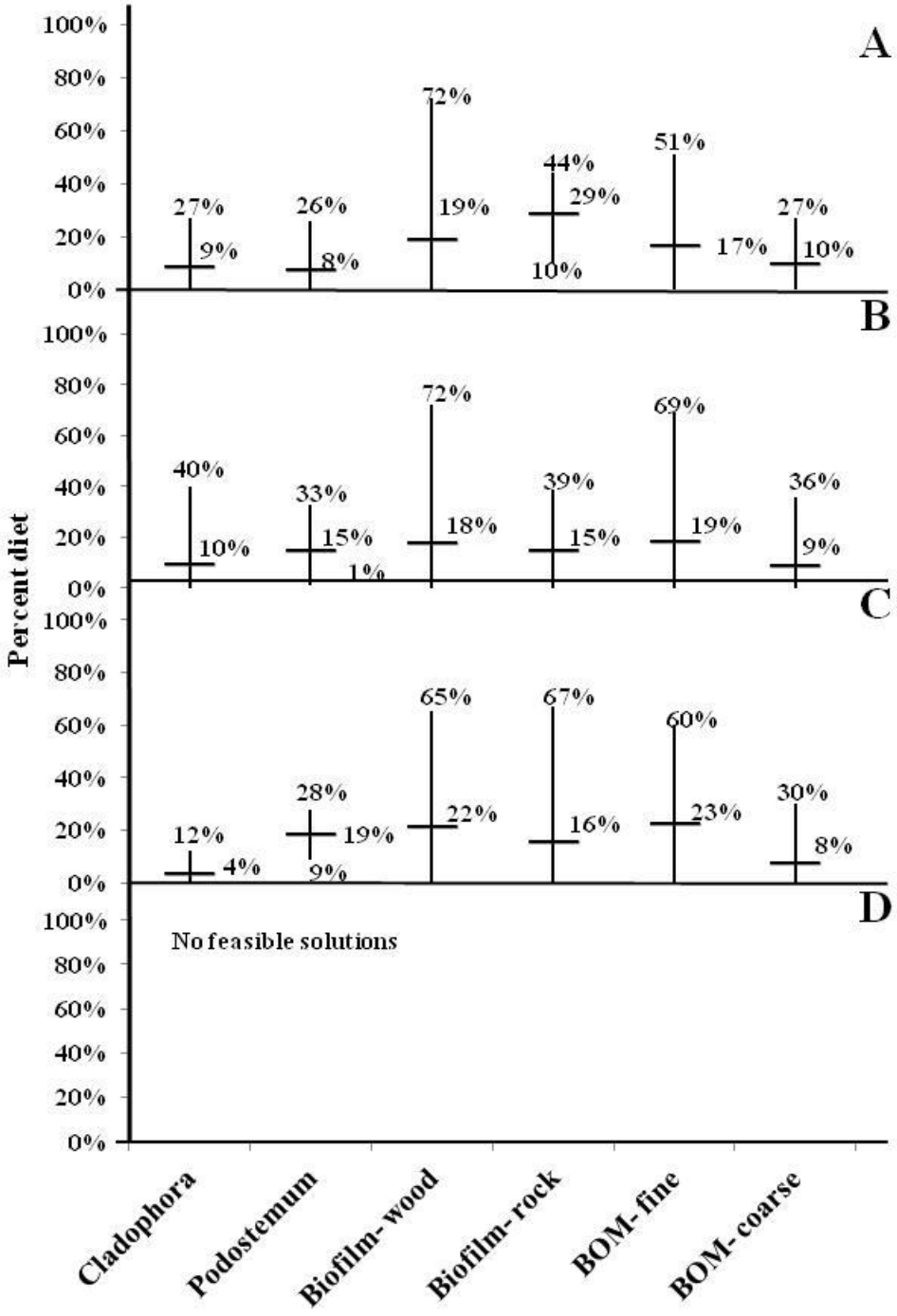

Figure 53: IsoSource results for *L. praerosa* and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

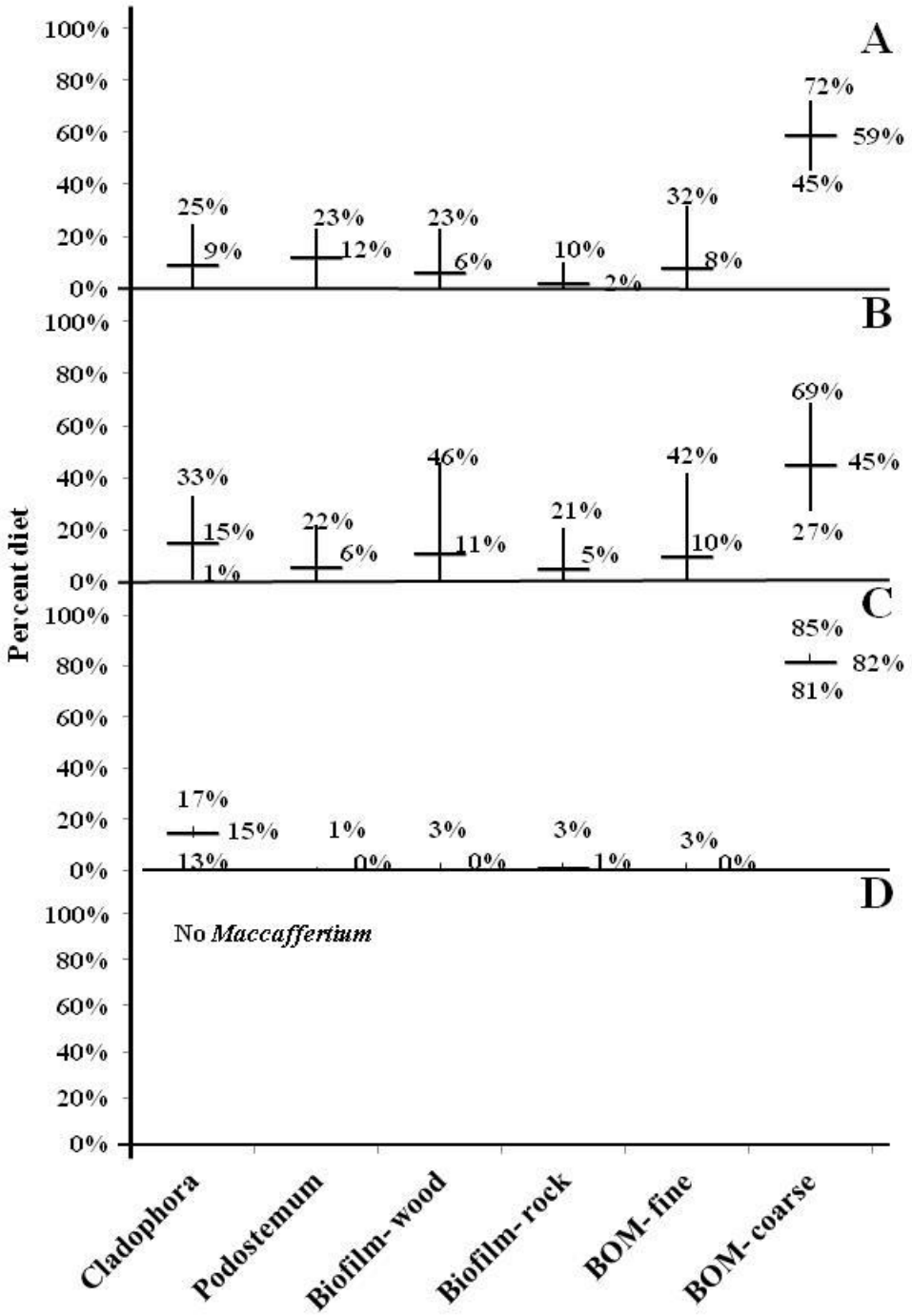

Figure 54: IsoSource results for *Maccaffertium mediopunctatum* and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

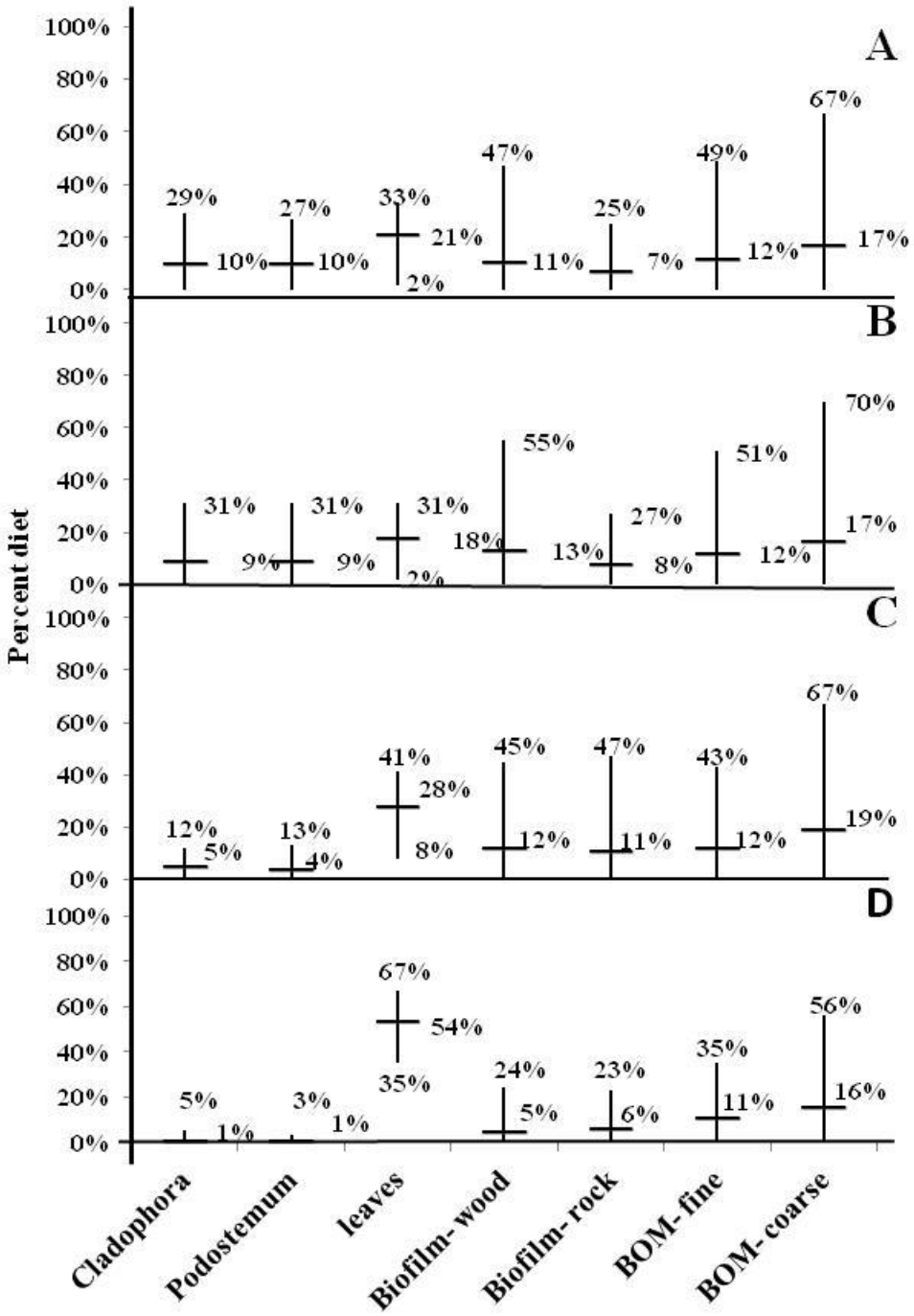

Figure 55: IsoSource results for *Optioservus* and seven potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

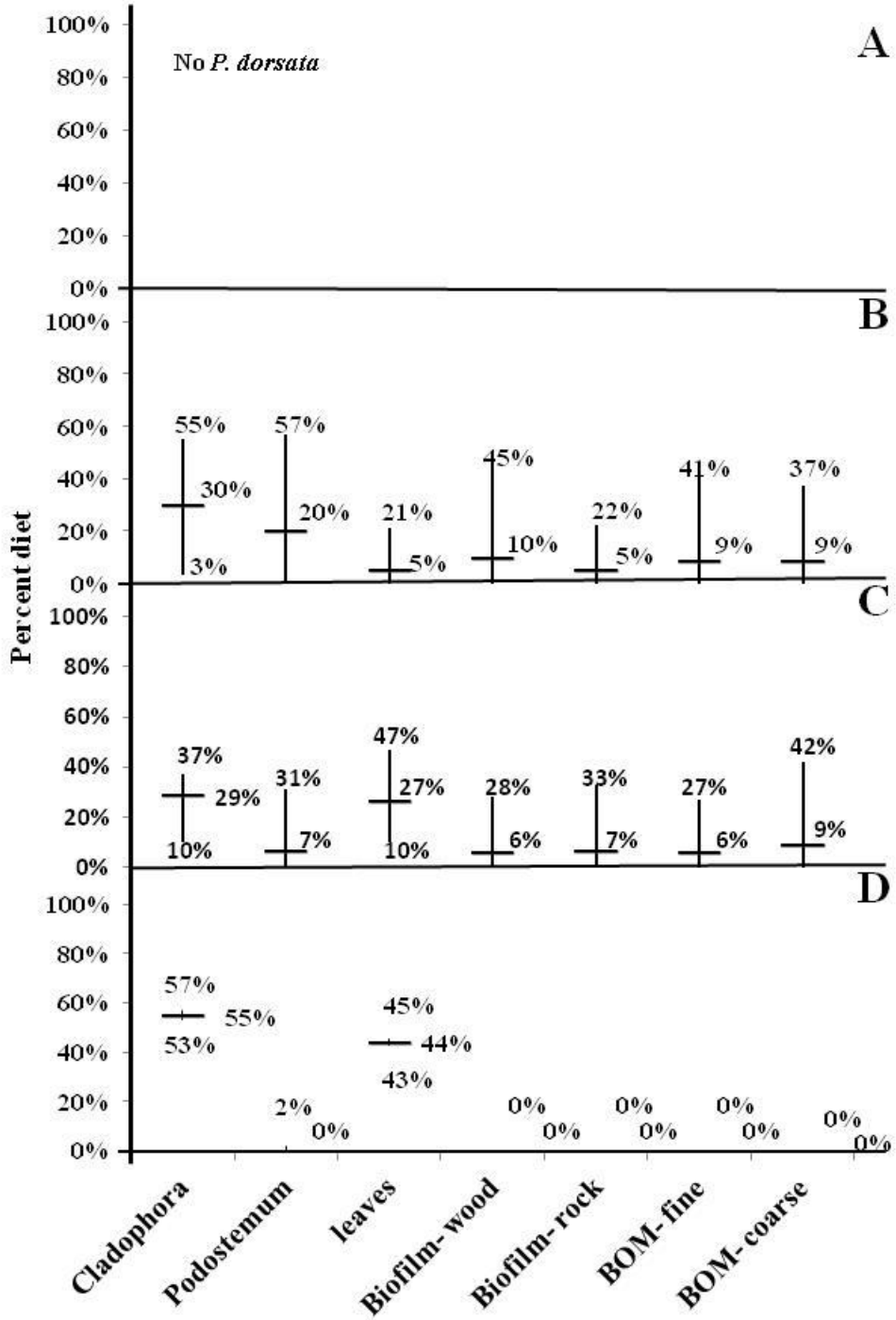

Figure 56: IsoSource results for *P. dorsata* and seven potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

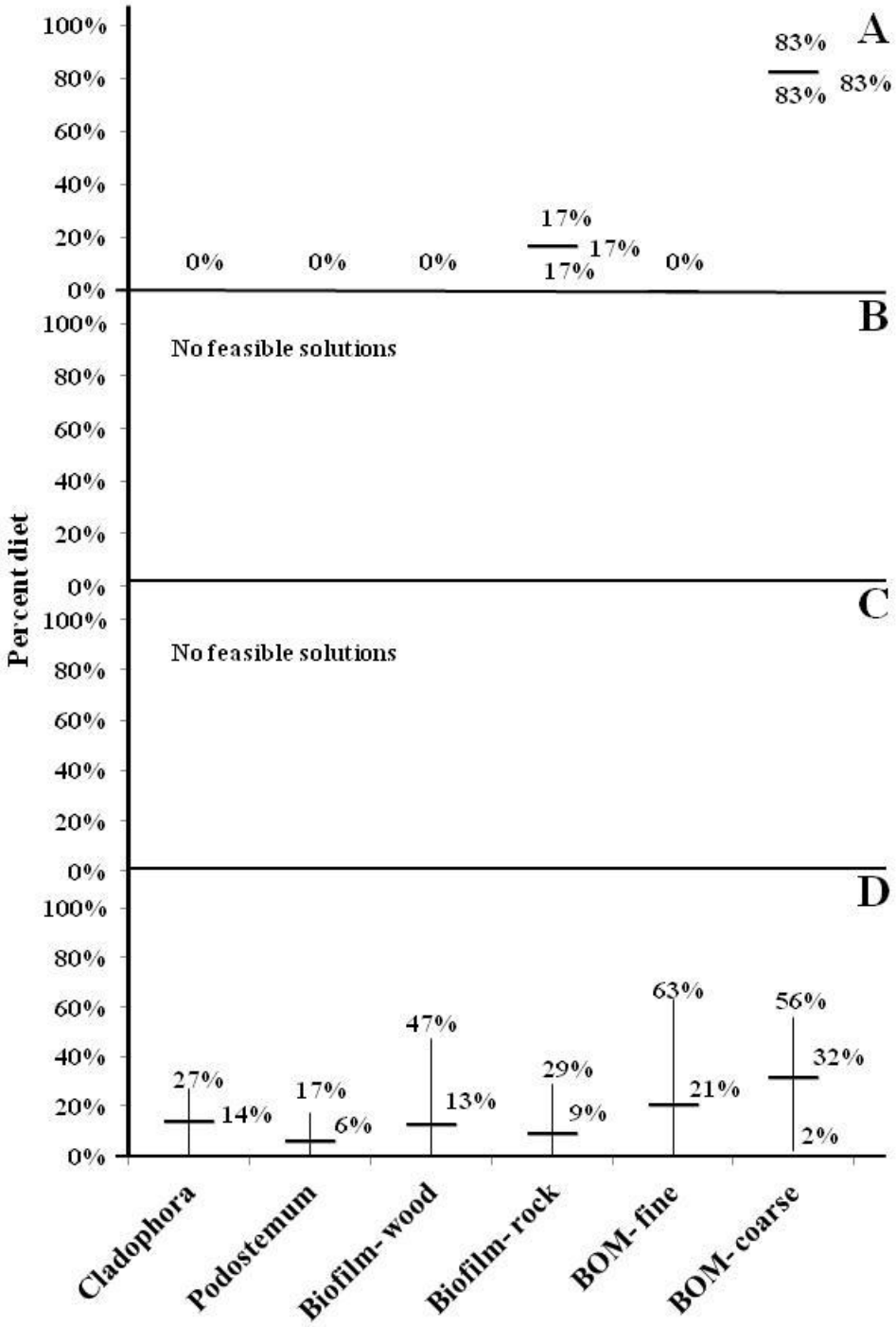

Figure 57: IsoSource results for *P. herricki* and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

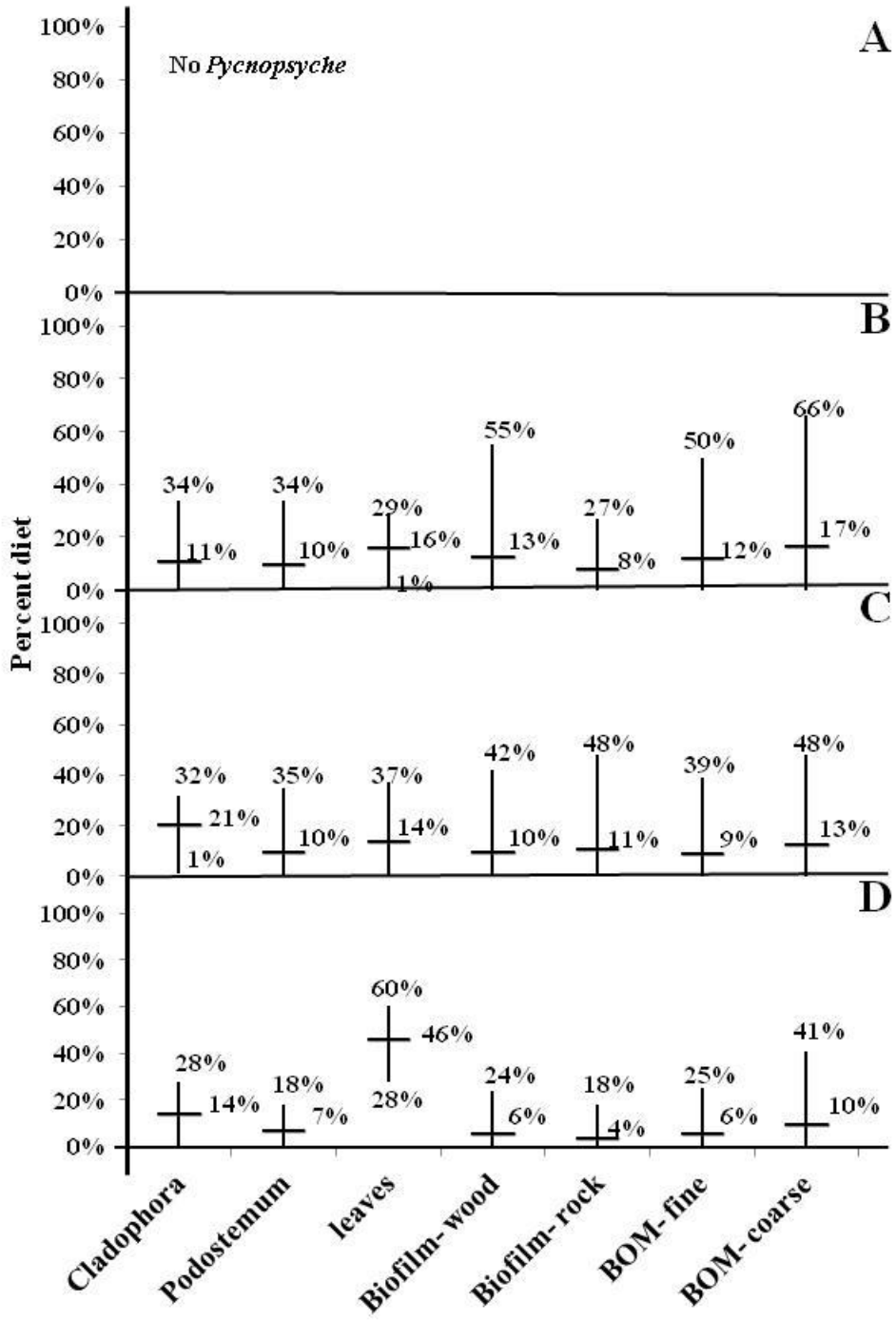

Figure 58: IsoSource results for *Pycnopsyche* and seven potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

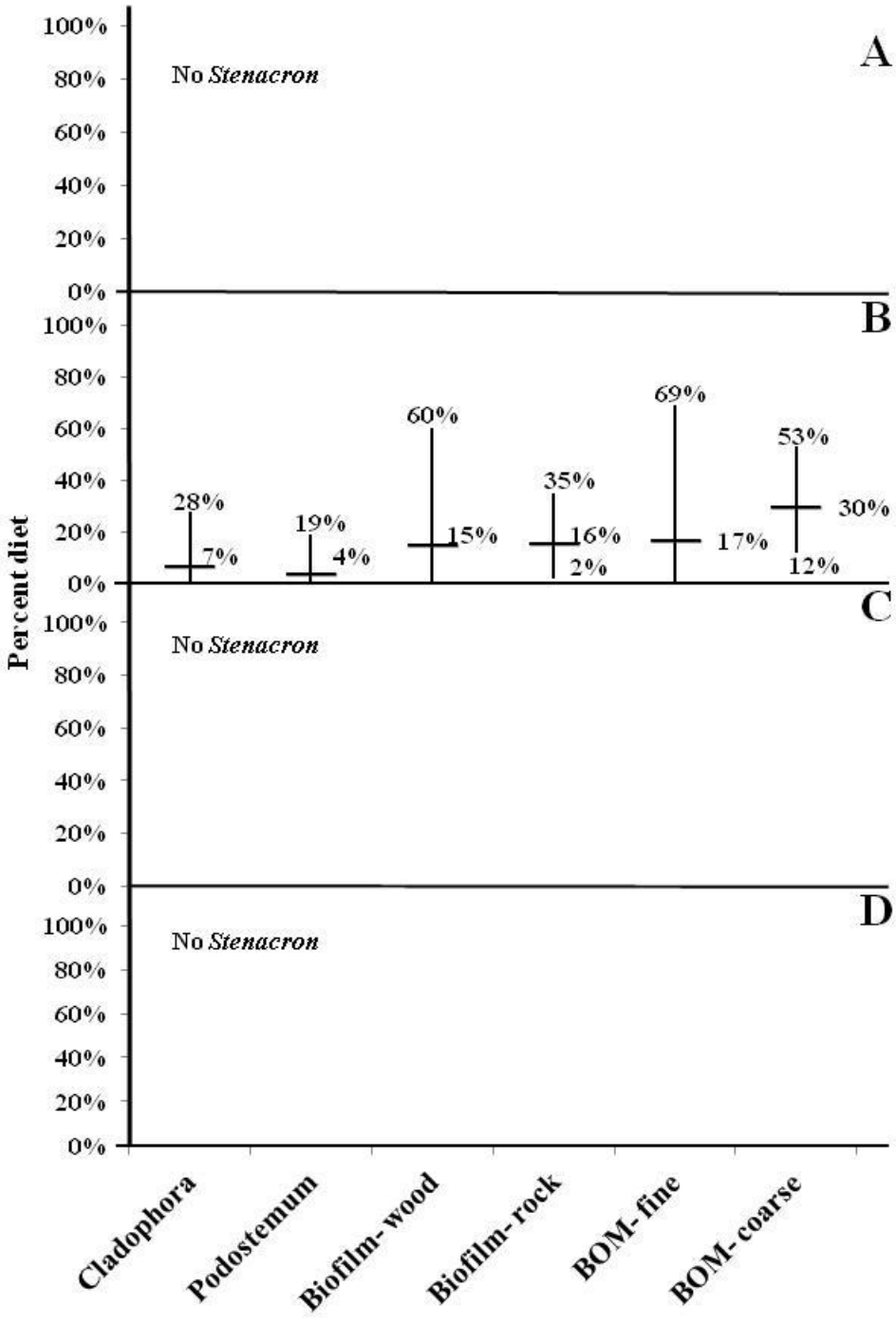

Figure 59: IsoSource results for *Stenacron* and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

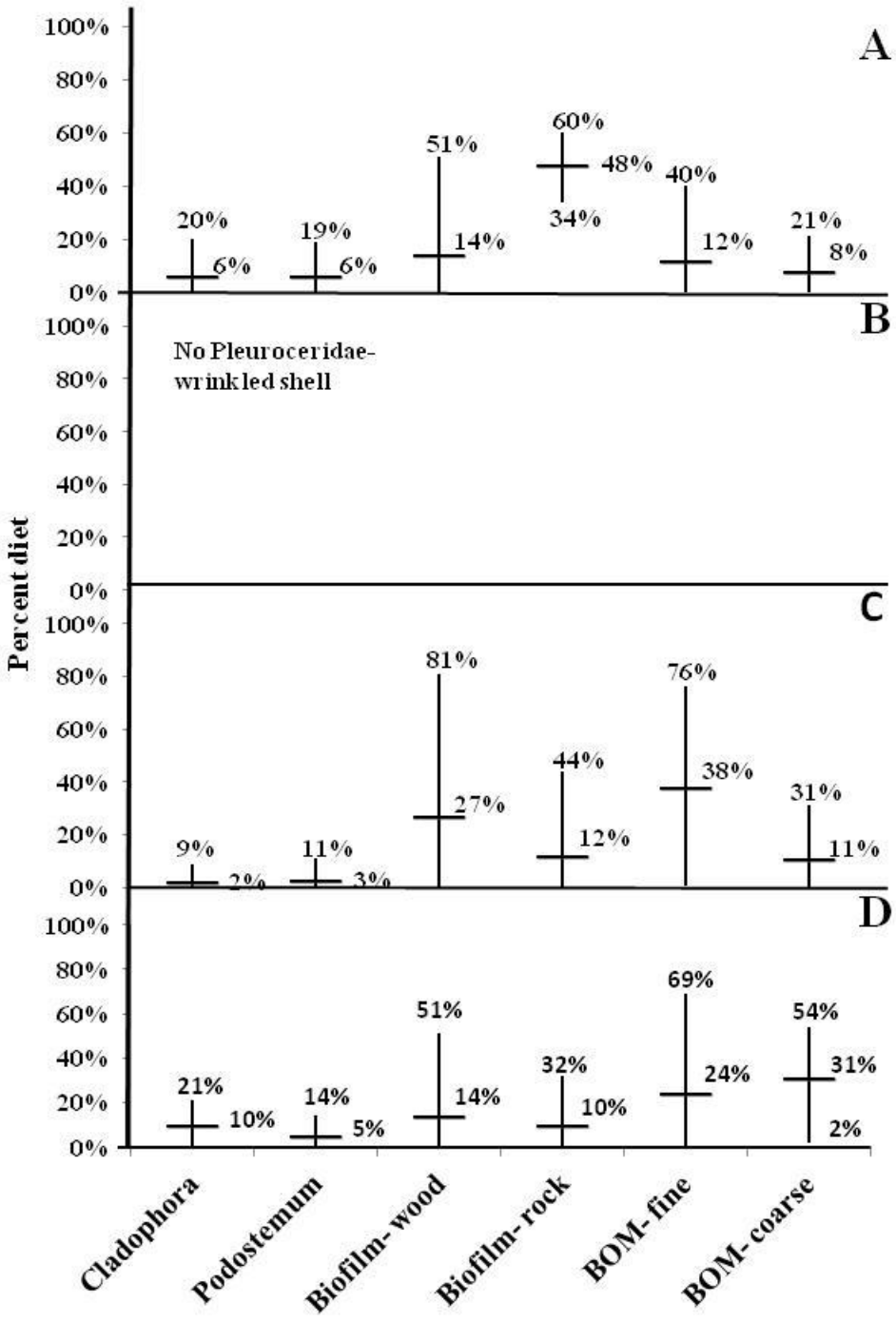

Figure 60: IsoSource results for Pleuroceridae-wrinkled shell and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

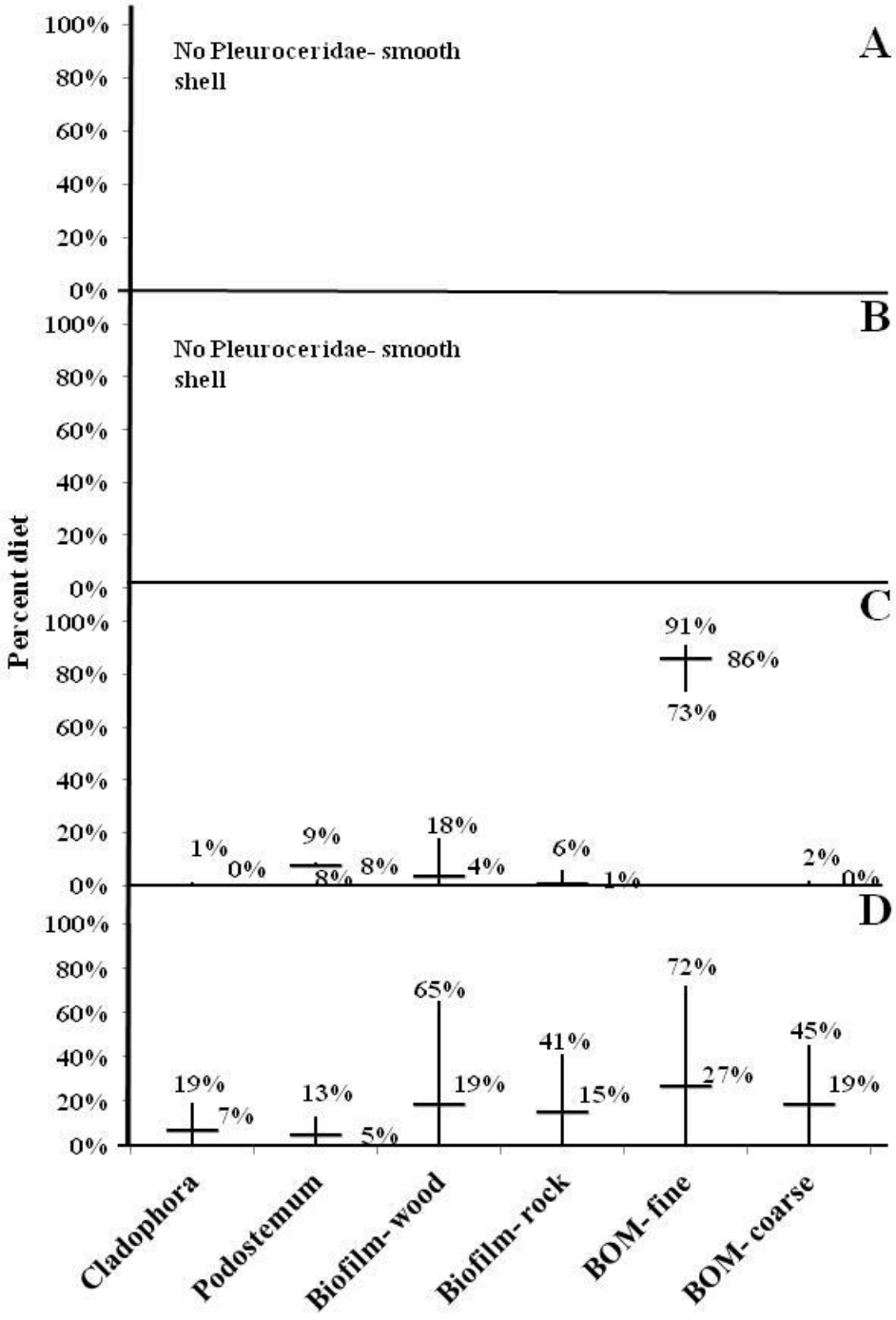

Figure 61: IsoSource results for Pleuroceridae-smooth shell and six potential food resources in four reaches along the upper Green River during autumn 2013. Sites A through D are in order from upstream to downstream. Ranges represent 1-99th percentile of feasible contributions of each source to the consumer's diet as calculated by IsoSource (Philips & Gregg 2003).

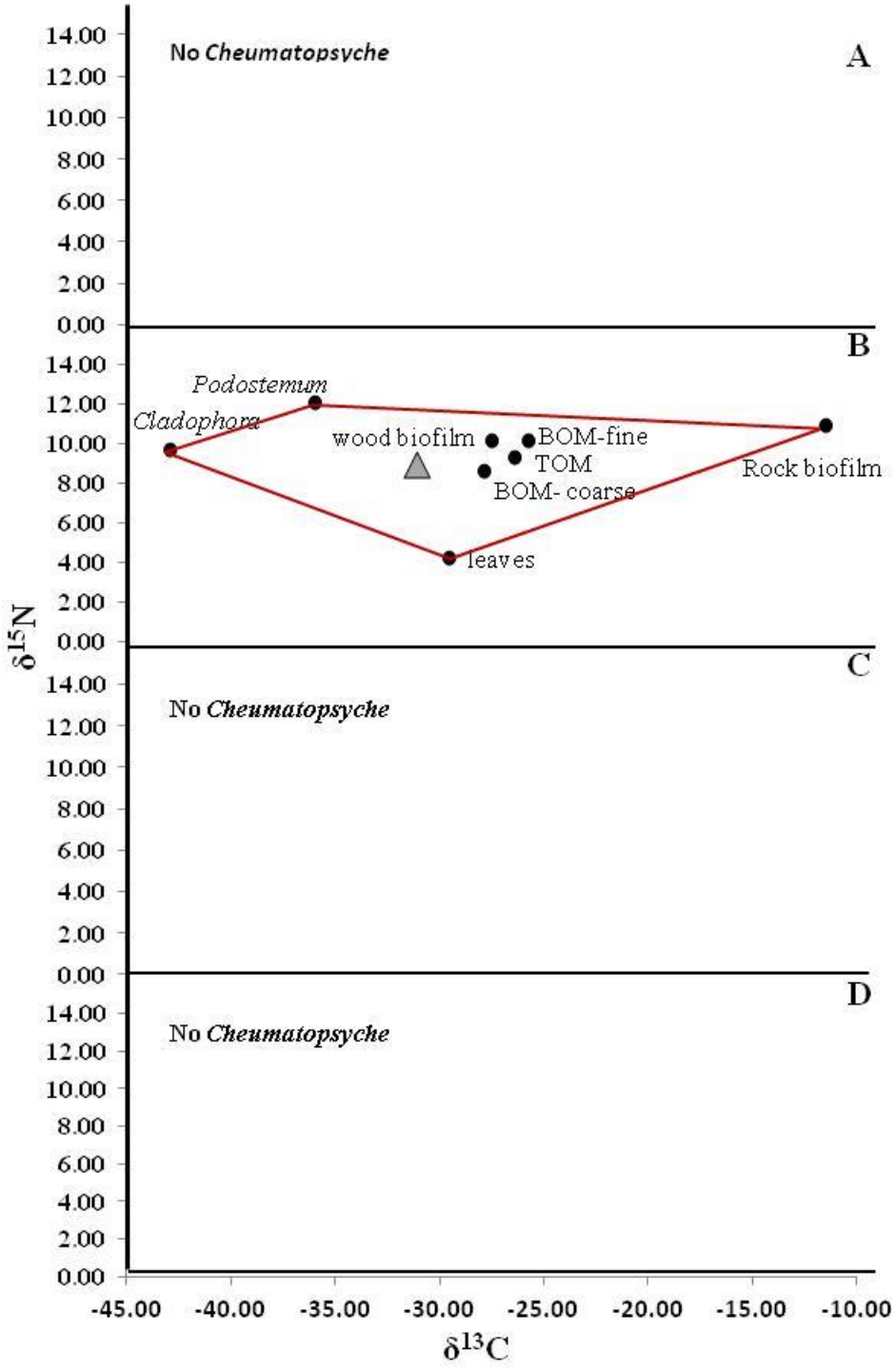

Figure 62: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Cheumatopsyche* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

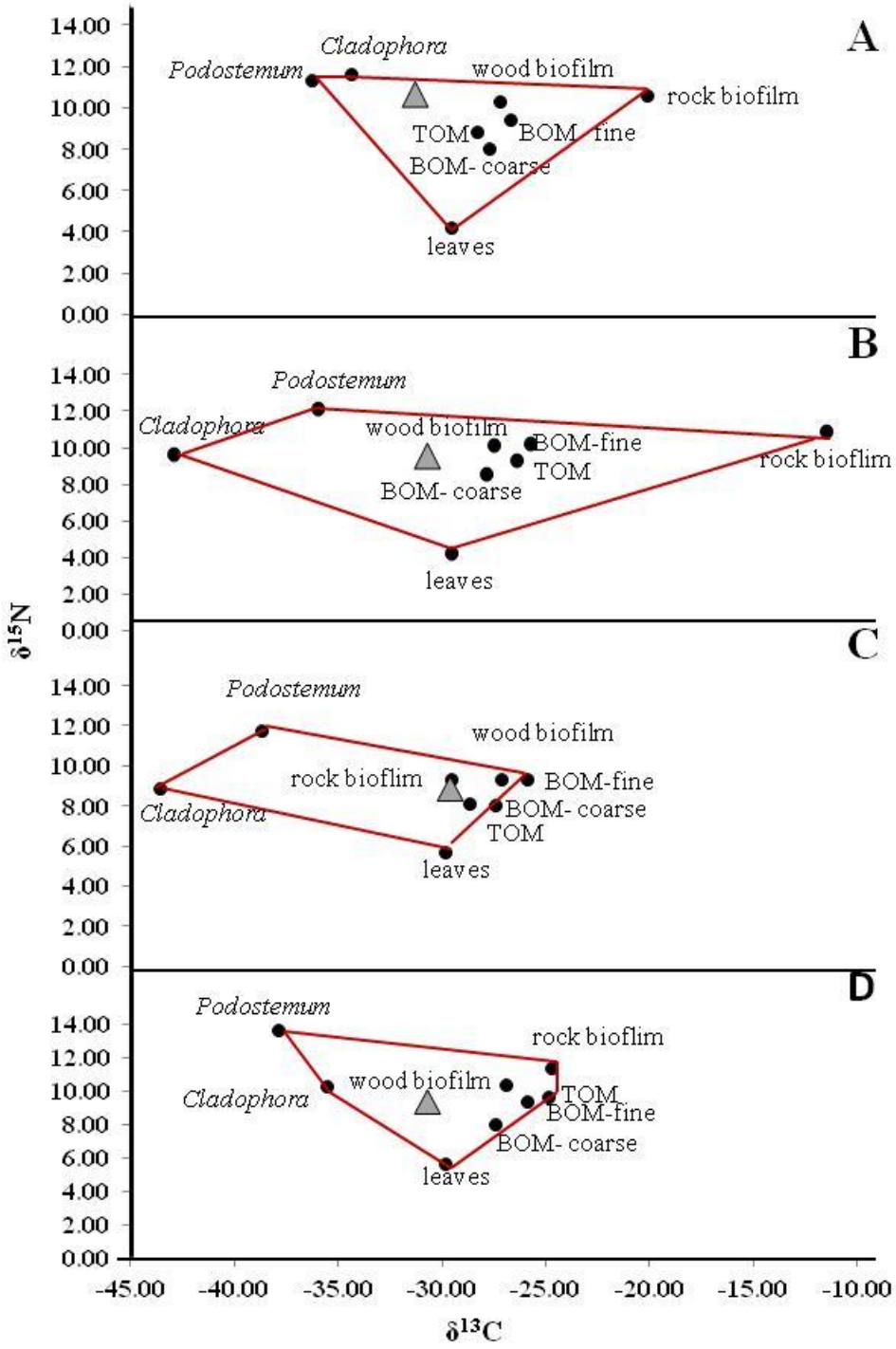

Figure 63: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *C. fluminea* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

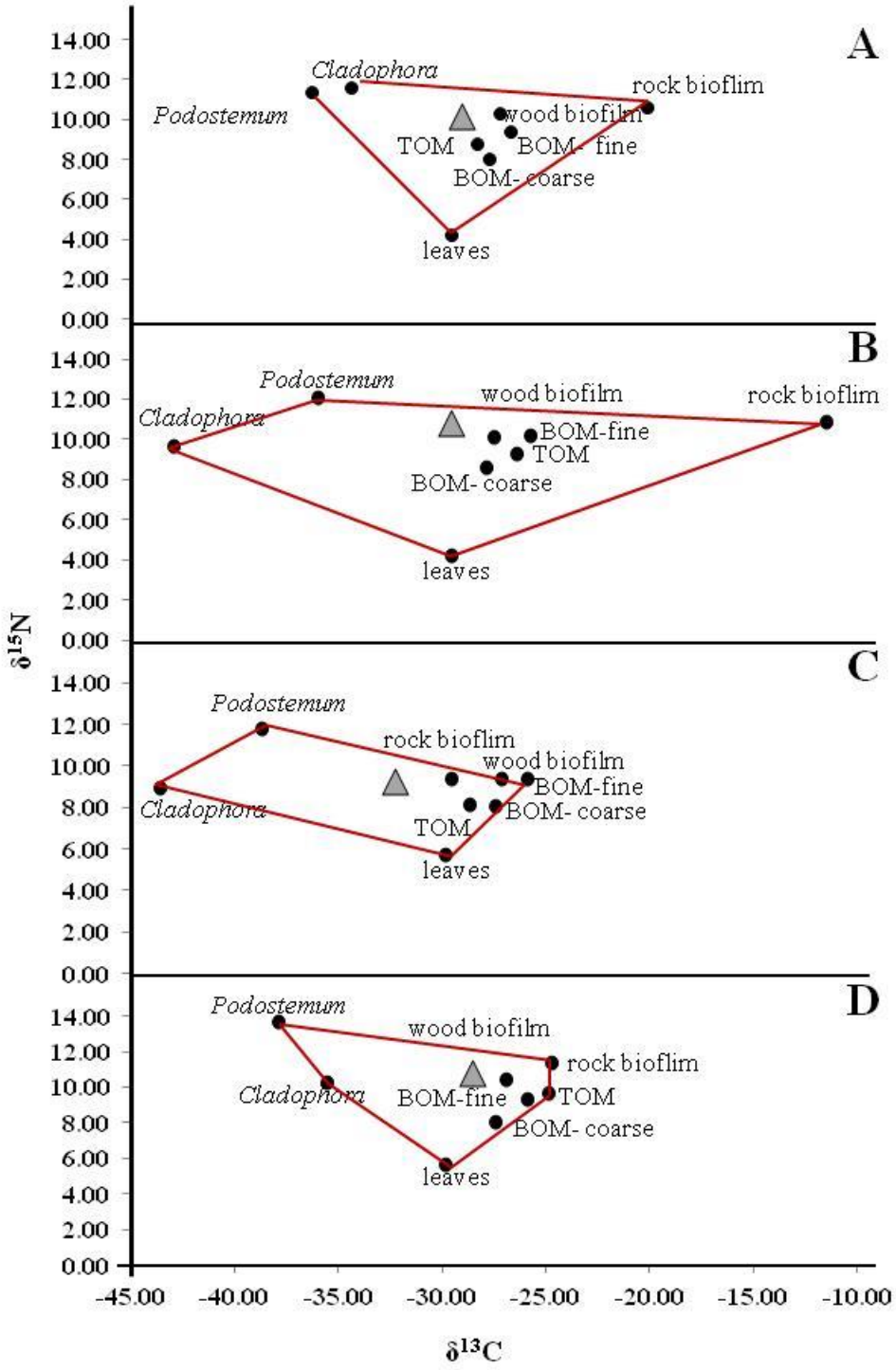

Figure 64: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *H. simulans* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

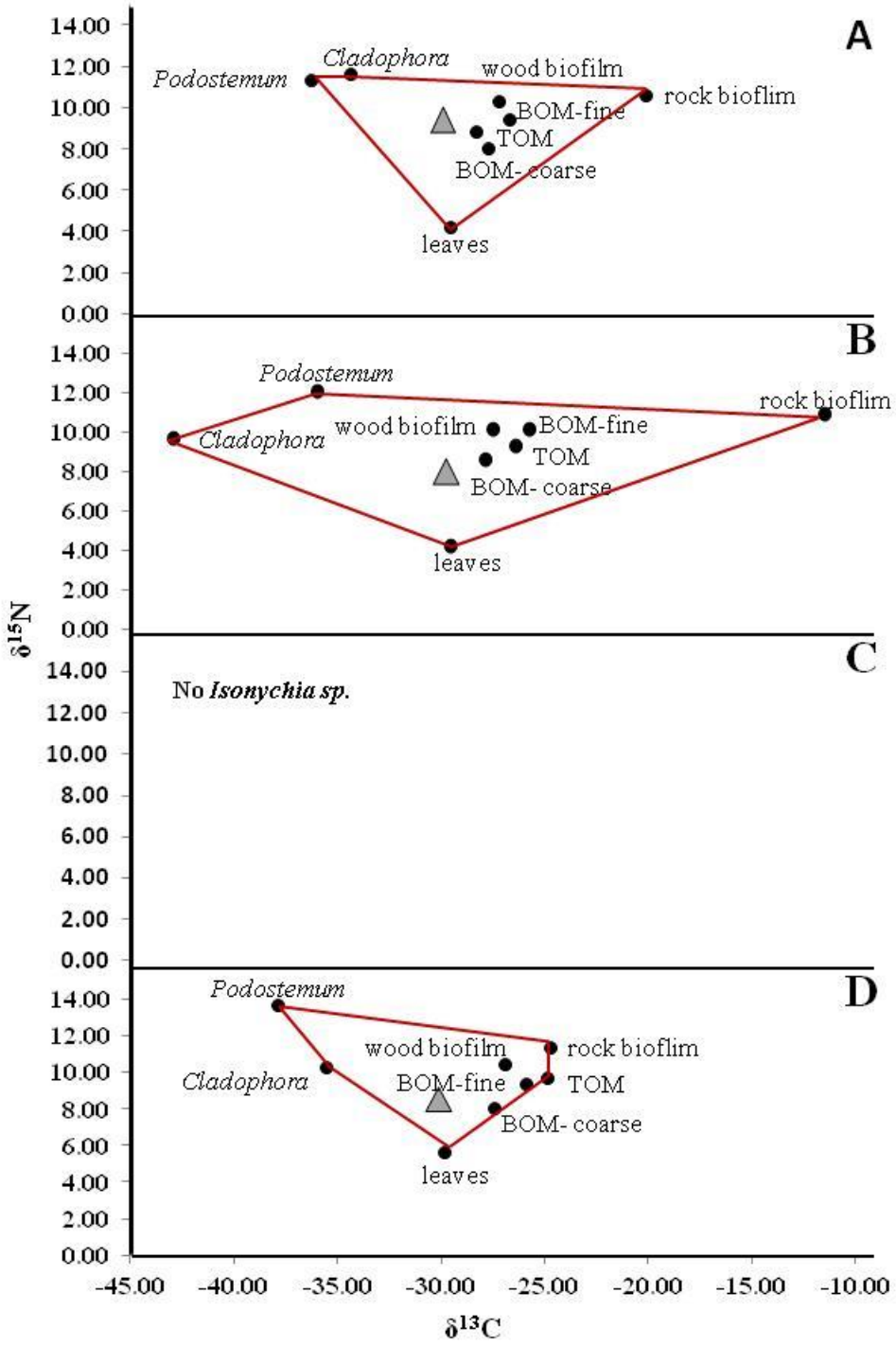

Figure 65: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Isonychia* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

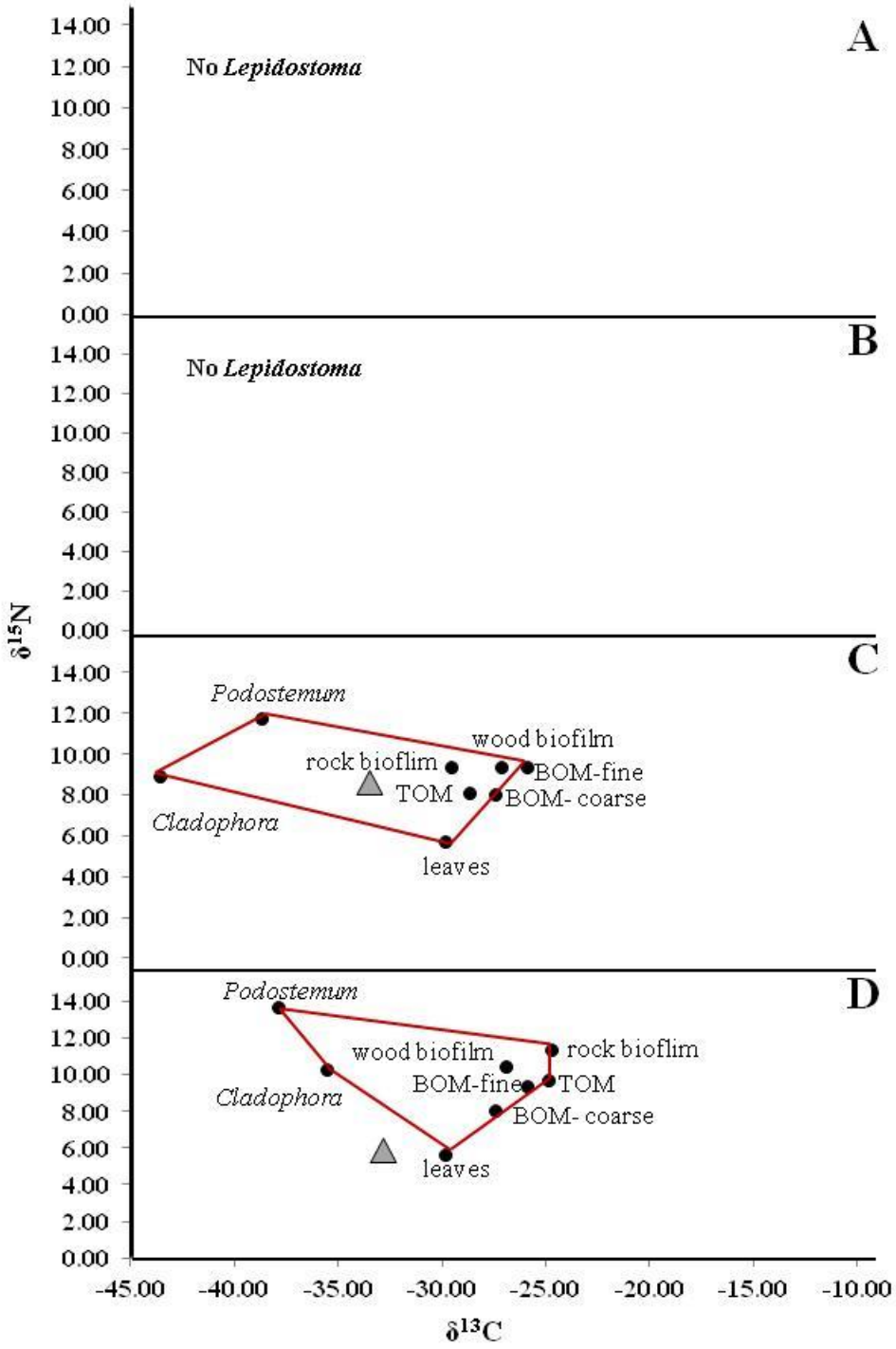

Figure 66: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Lepidostoma* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

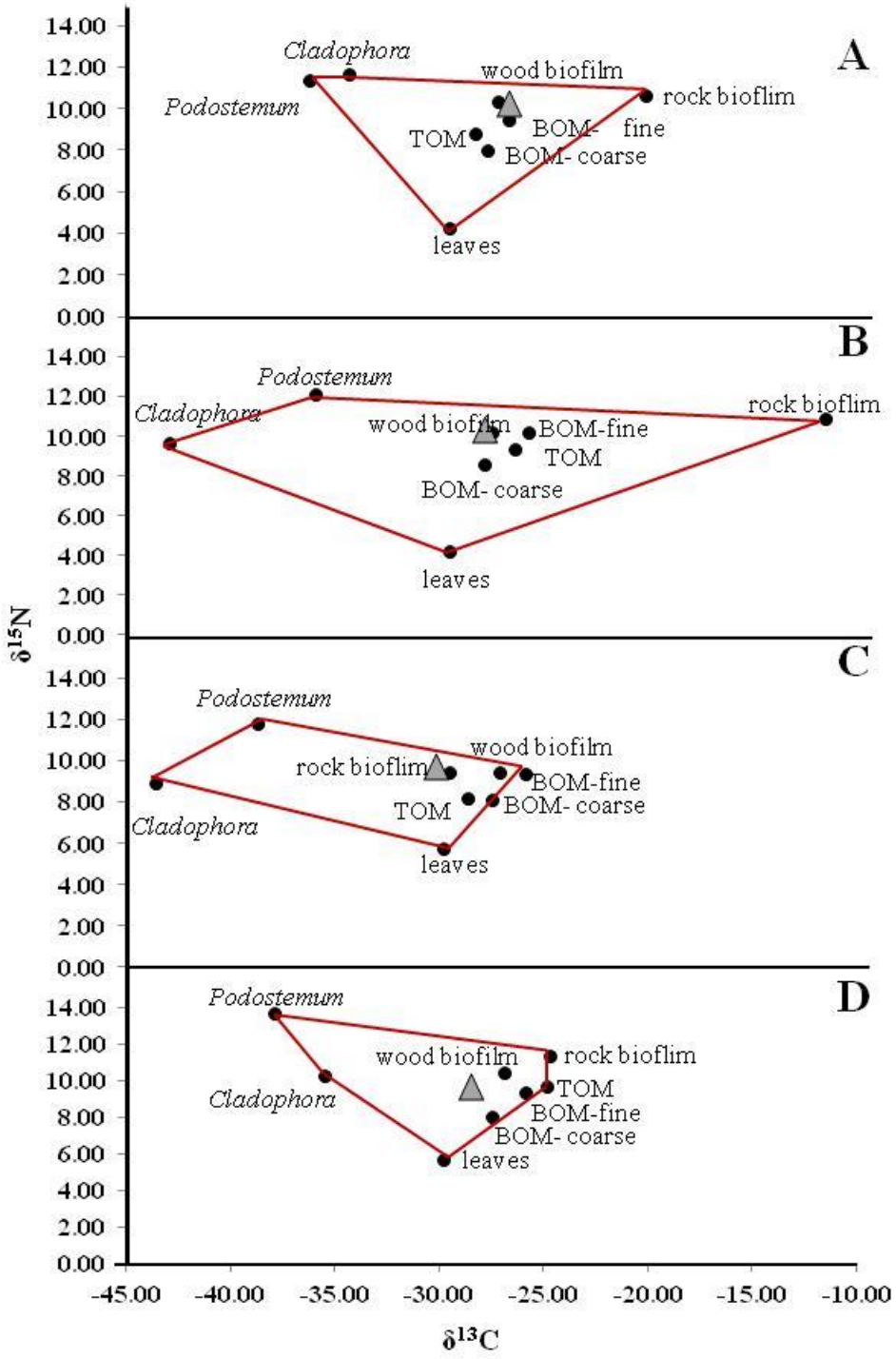

Figure 67: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *L. praerosa* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

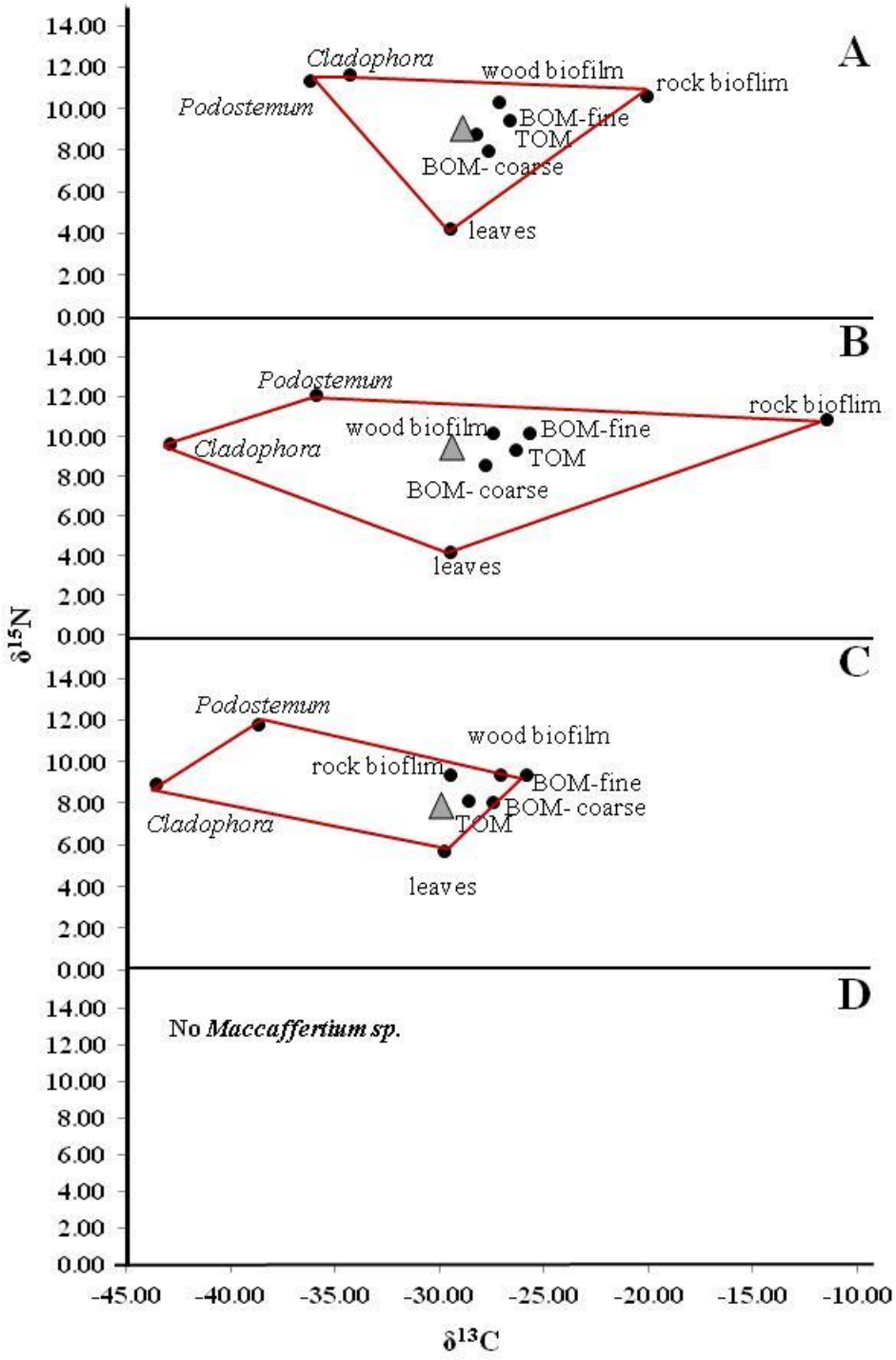

Figure 68: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Maccaffertium mediopunctatum* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

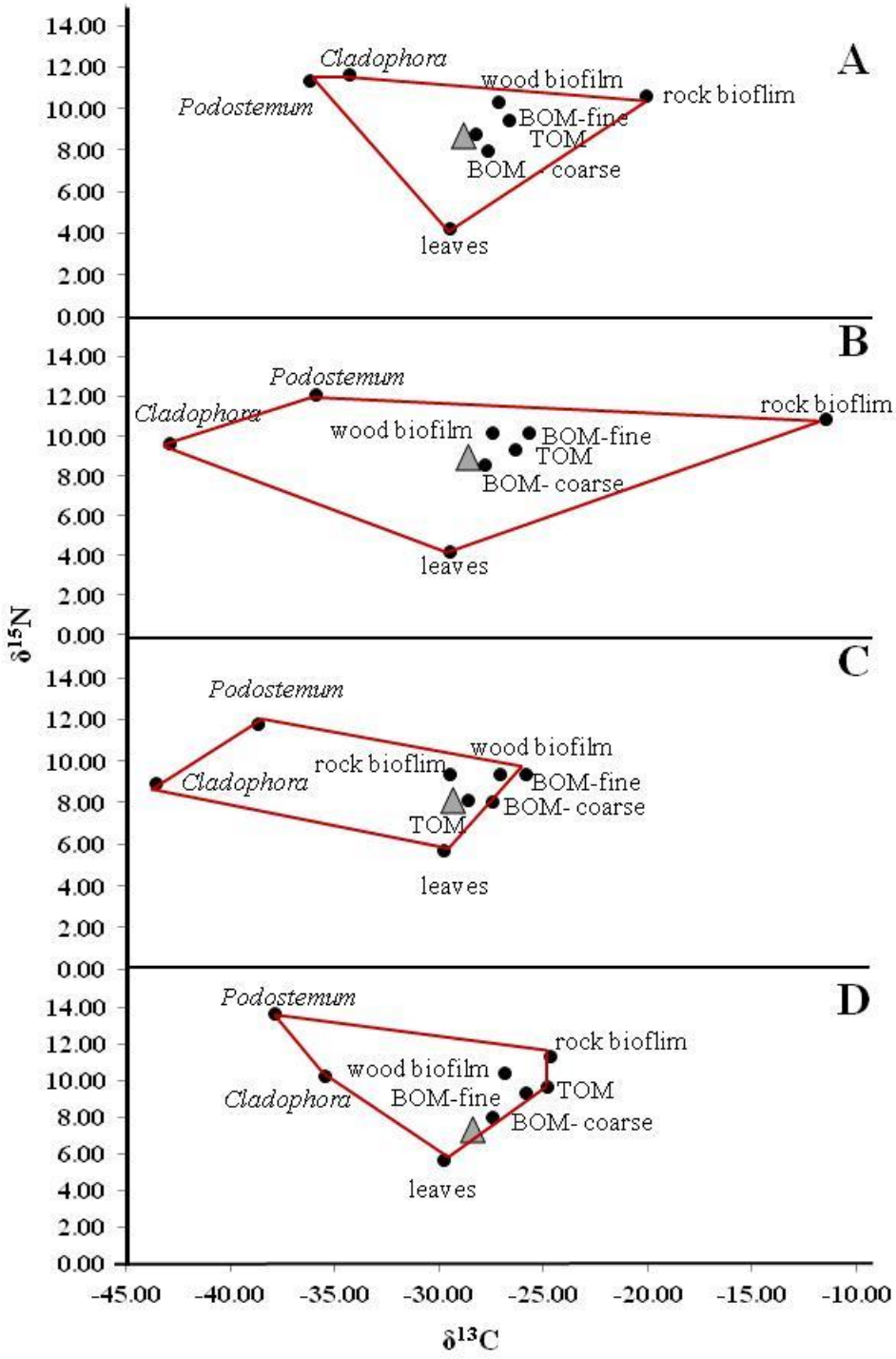

Figure 69: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Optioservus* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

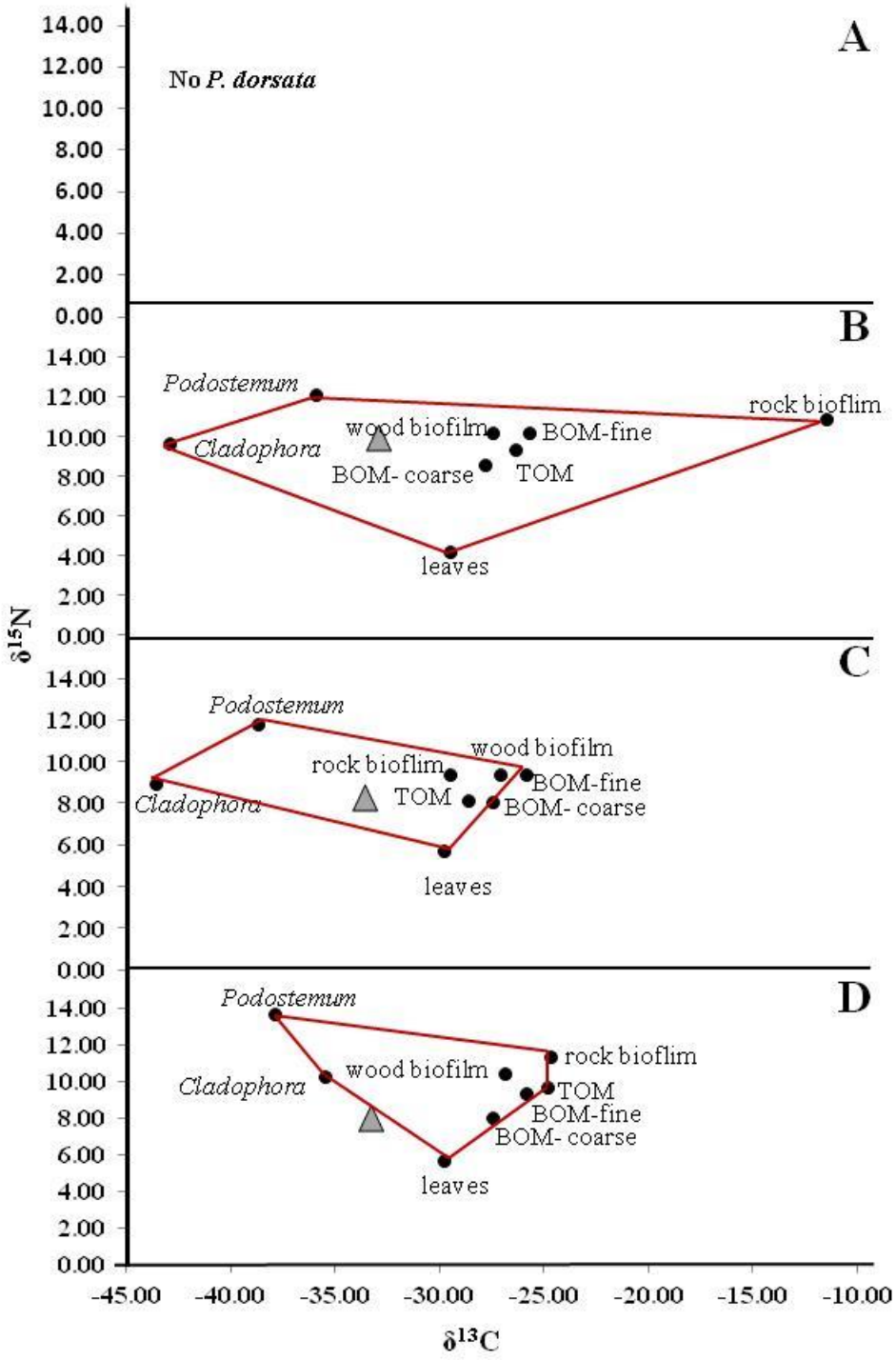

Figure 70: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *P. dorsata* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

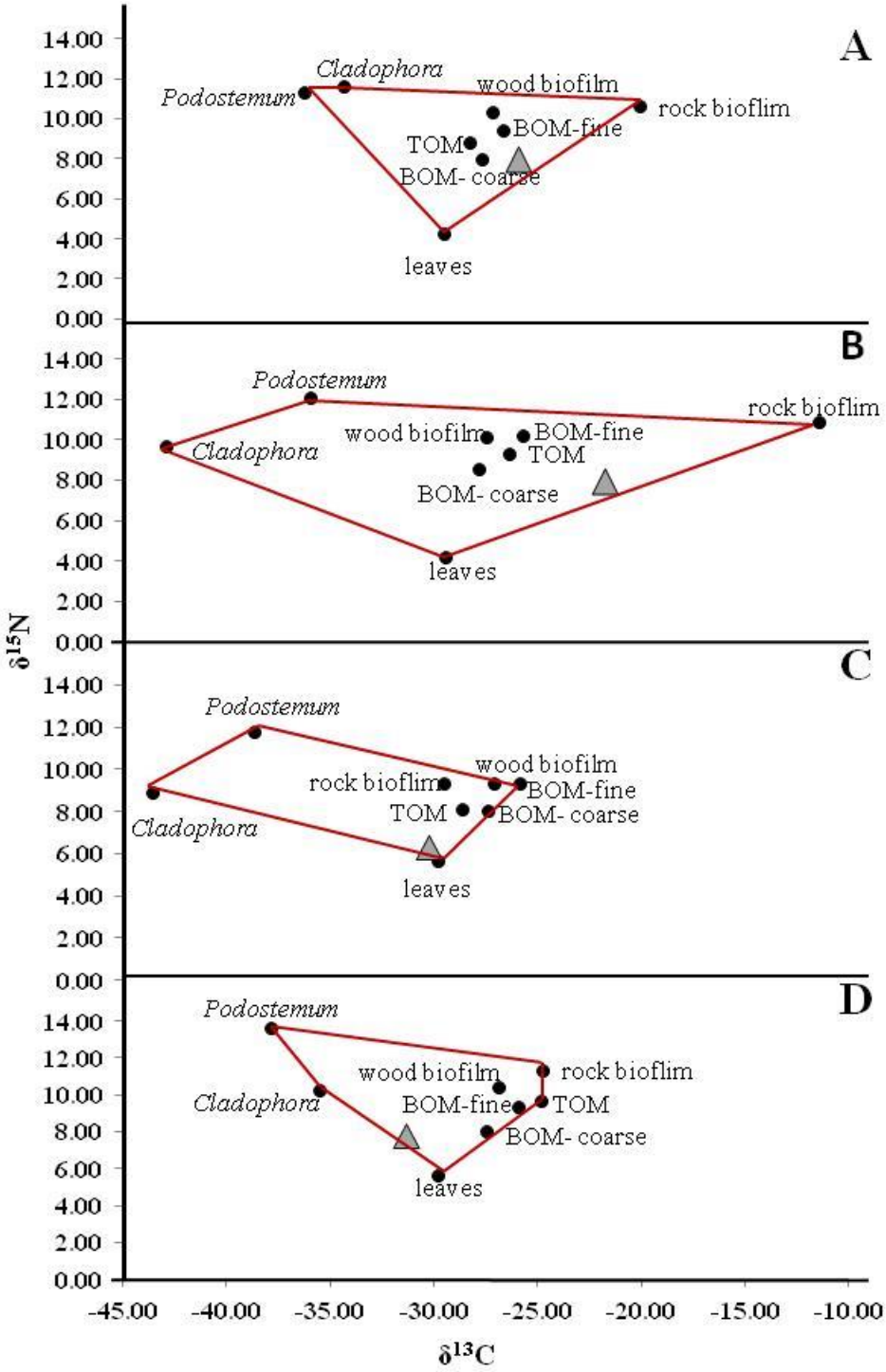

Figure 71: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *P. herricki* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

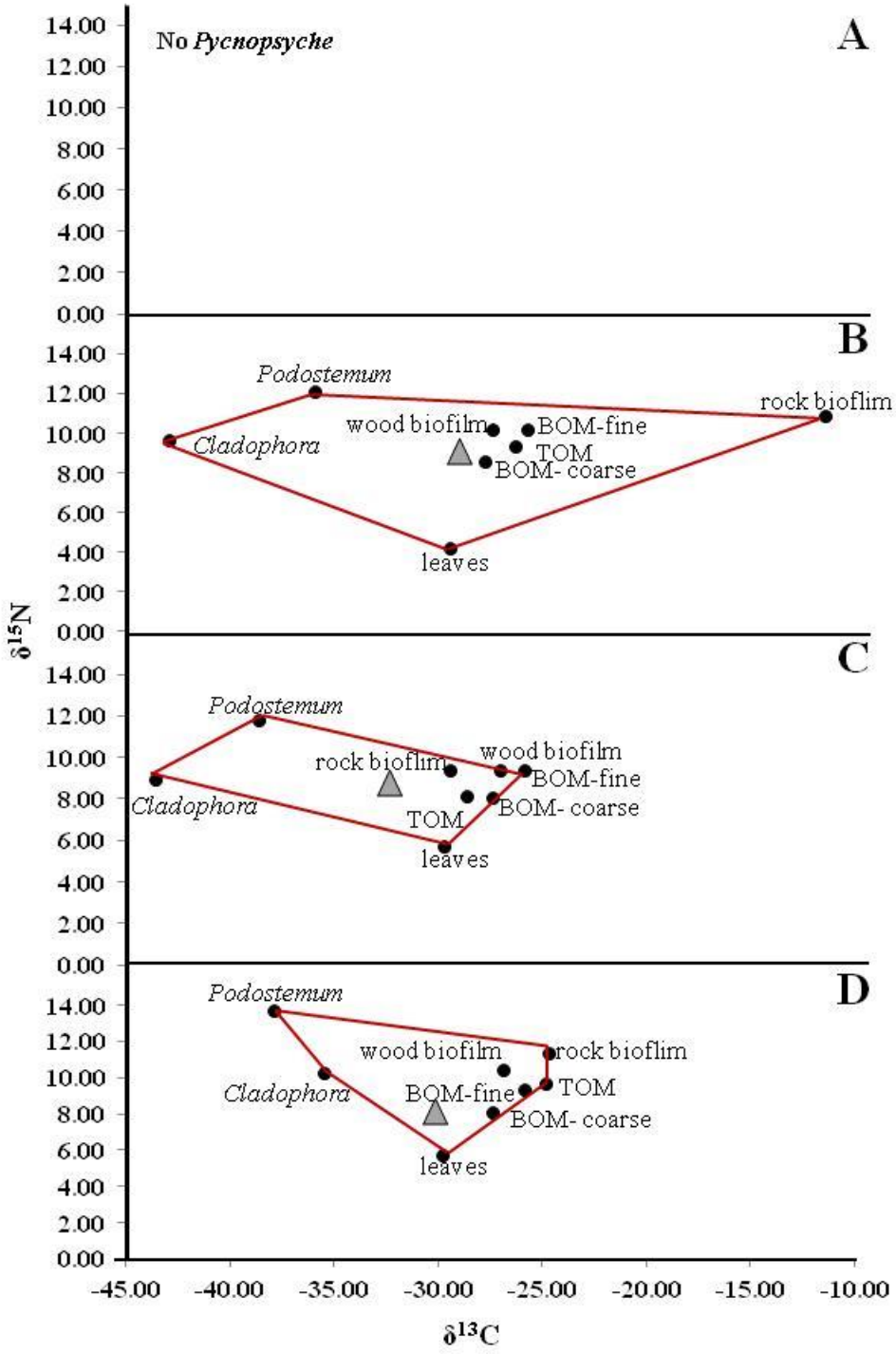

Figure 72: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Pycnopsyche* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

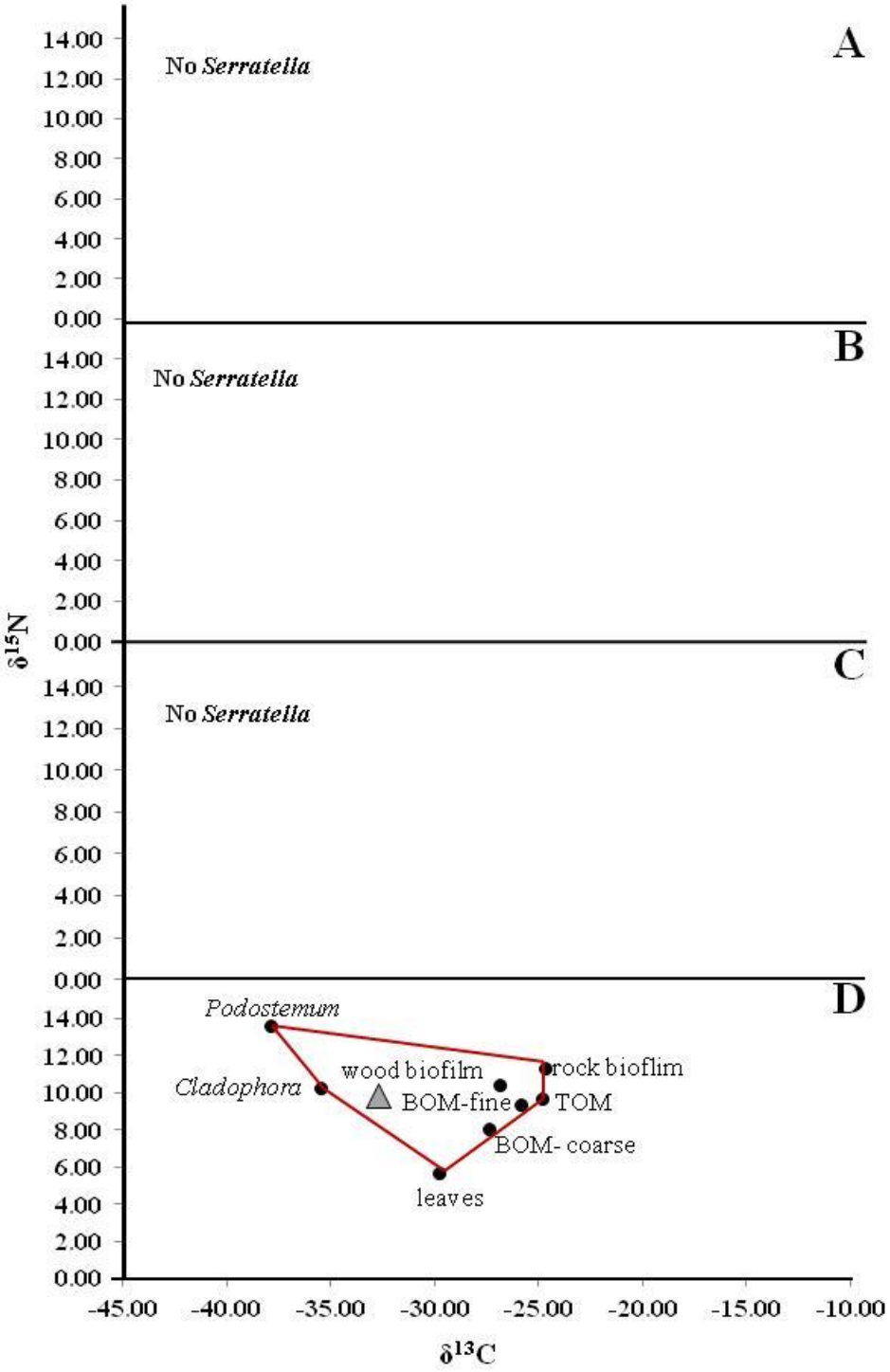

Figure 73: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Serratella* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

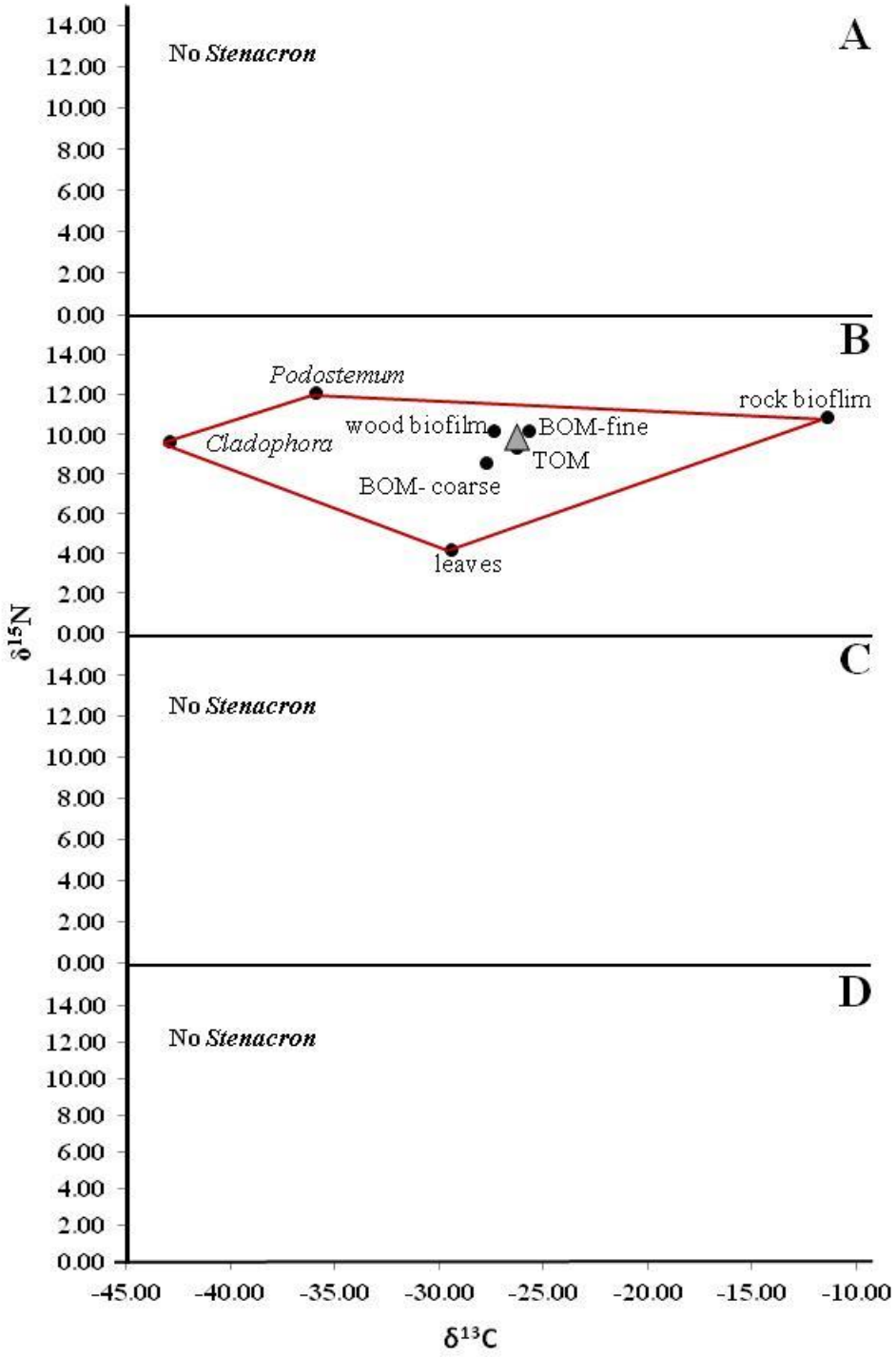

Figure 74: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *Stenacron* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

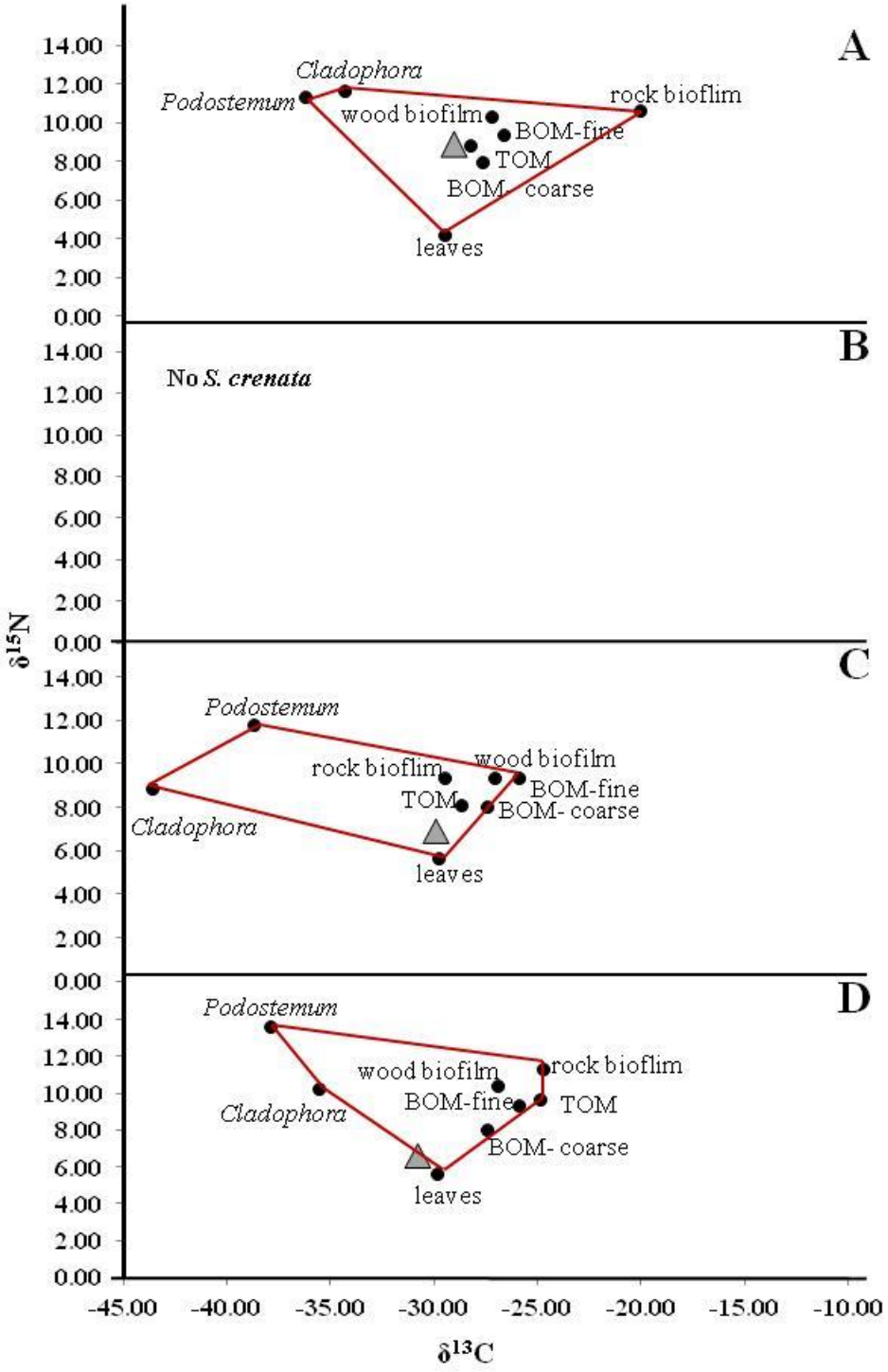

Figure 75: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of *S. crenata* and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

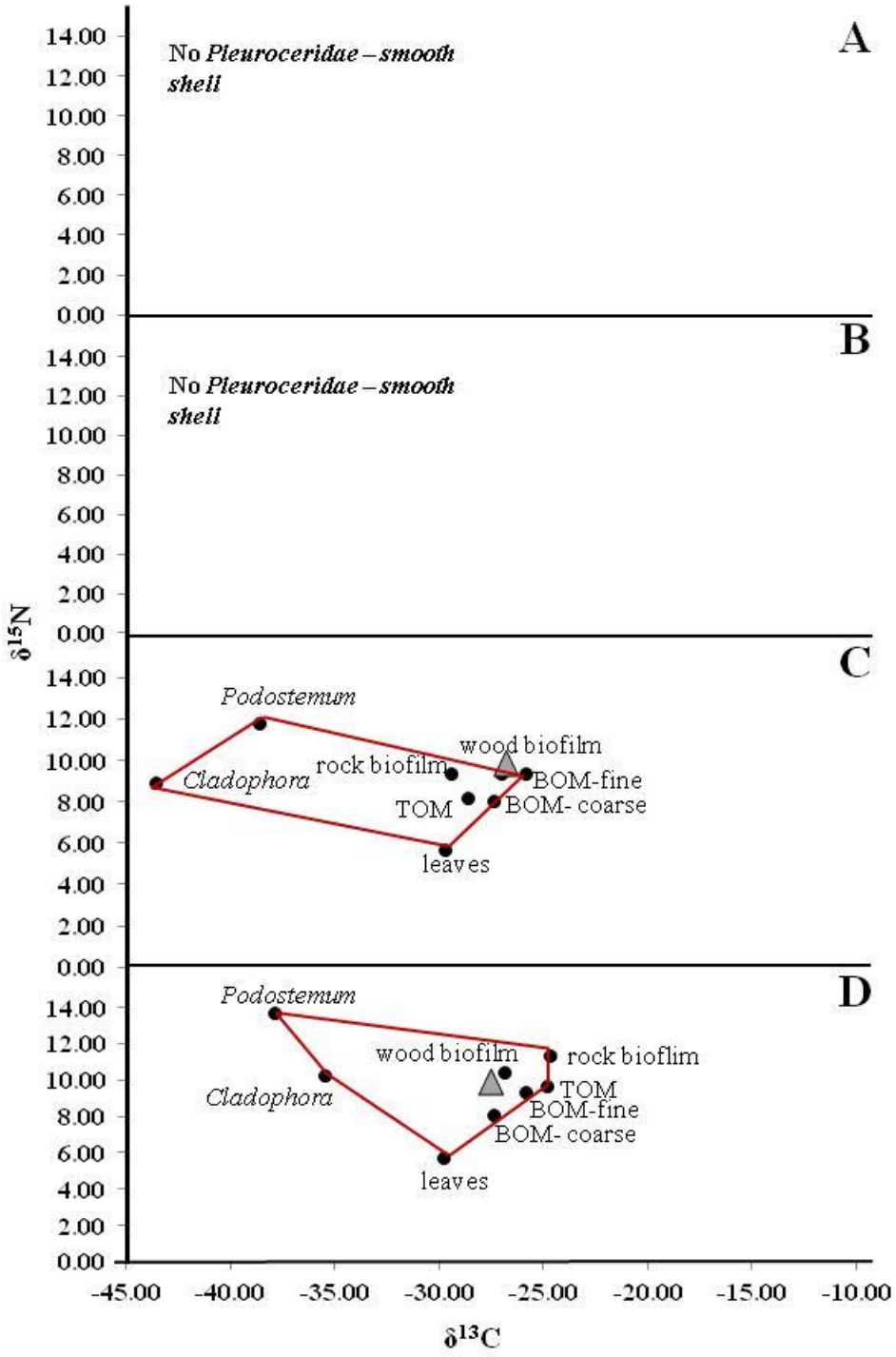

Figure 76: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of Pleuroceridae-smooth shell and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

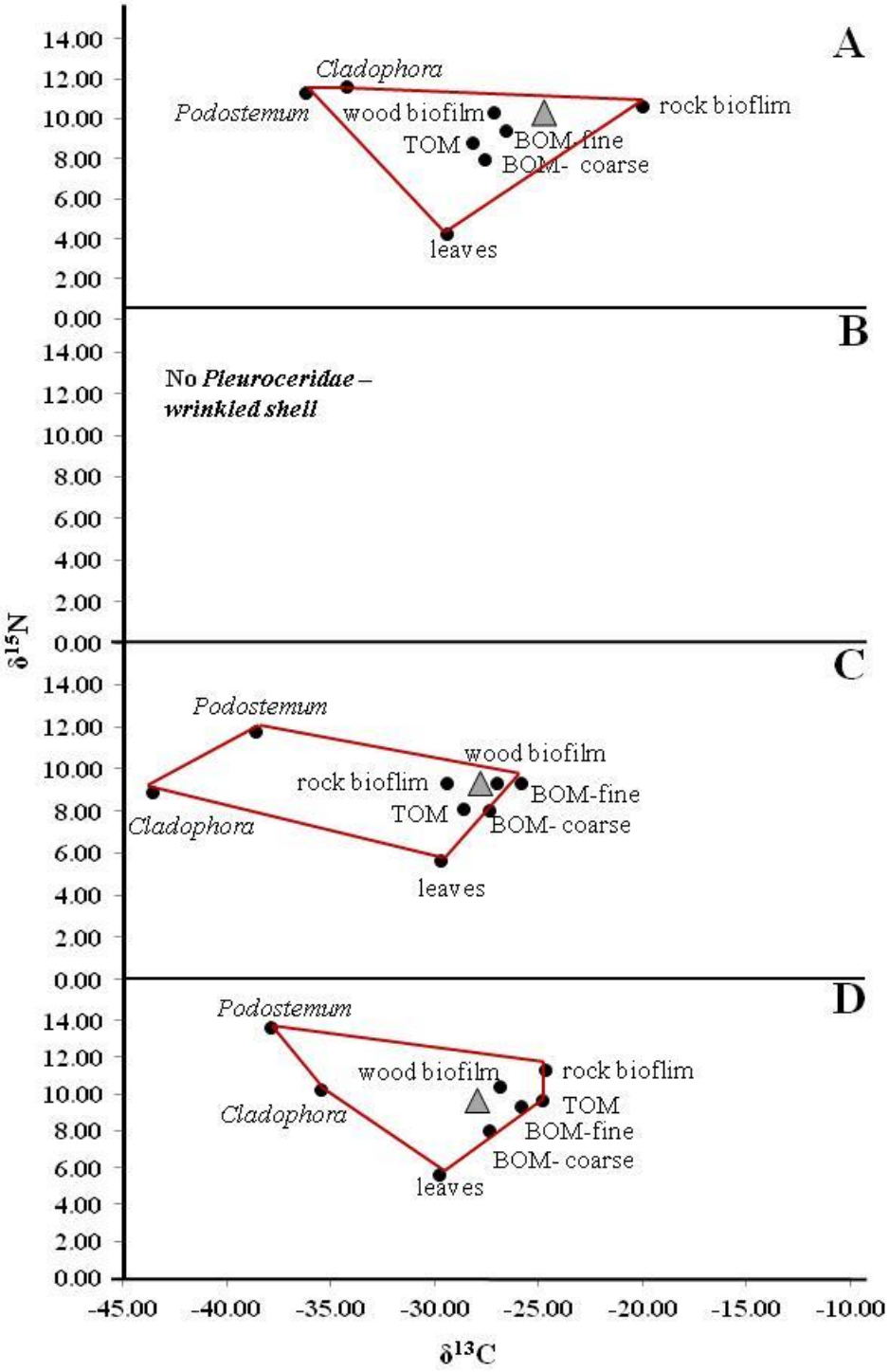

Figure 77: Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of Pleuroceridae-wrinkled shell and eight potential food sources during autumn 2013. Sites A through D are the four study reaches along the upper Green River are in order from upstream to downstream.

Table 1: Carbon and nitrogen isotope data from potential food resources collected from the upper Green River during summer 2012. Reaches are listed in order of upstream to downstream. n.a. = resources not collected from a study reach.

Isotopic ratio	**Reach**			
$\delta^{13}C$	A	B	C	D
Cladophora	-18.53 ± 0.04	-25.80 ±0.13	-29.95 ± 0.19	-32.44 ± 0.10
P. ceratophyllum	-29.39 ± 0.02	-26.5 ± 0.27	-35.00 ± 0.04	-38.81
epilithic bioflim - detrital	-19.58	-15.91	-15.36	-25.18
epilithic biofilm - algal	-18.62	n.a.	-25.20	-27.91
composite TOM	-25.07	-24.65	-26.93	n.a.
DOM	-25.22	-7.15	-5.09	-25.80
P. occidentalis	-30.65 ± 0.01	-30.64 ± 0.11	-30.12 ± 0.02	-30.50 ± 0.03
A. saccharinum	-28.47 ± 0.04	-29.74 ± 0.03	-30.04 ± 0.04	-31.76 ± 0.04
A. negundo	-30.78 ± 0.05	-31.76 ± 0.03	-30.89 ± 0.04	-30.77 ± 0.04
$\delta^{15}N$				
Cladophora	7.87 ± 0.02	6.82 ± 0.05	5.81 ± 0.06	4.84 ± 0.09
P. ceratophyllum	6.85 ± 0.18	7.29 ± 0.19	6.86 ± 0.04	8.03
epilithic bioflim - detrital	6.95	6.99	6.00	5.92
epilithic biofilm - algal	6.23	n.a.	2.76	5.88
composite TOM	3.40	3.42	2.16	n.a.
DOM	5.84	5.10	7.38	15.52
P. occidentalis	-0.07 ± 0.12	2.72 ± 0.08	4.58 ± 0.03	4.80 ± 0.02
A. saccharinum	3.98 ± 0.02	2.42 ± 0.04	3.54 ± 0.04	2.82 ± 0.05
A. negundo	2.59 ± 0.02	1.54 ± 0.10	2.84 ± 0.06	3.15 ± 0.08

TOM = transported organic matter, DOM = dissolved organic matter

Table 2: Carbon and nitrogen isotope data from common primary consumer taxa collected from the upper Green River during summer 2012. Reaches are listed in order of upstream to downstream. n.a. = resources not collected from a study reach.

Isotopic ratio	**Reach**			
$\delta^{13}C$	A	B	C	D
Cheumatopsyche sp.	-26.09	-27.02	-29.81	n.a.
Corbicula fluminea	-30.90	-29.65	-30.69	-30.60
Hydropsyche simulans	-24.04	-26.70	-30.70	-29.56
Isonychia sp.	-26.12	-26.87	-31.34	-30.61
Lepidostoma sp.	-29.94	-28.82	-29.90	n.a.
Leptoxis praerosa	-27.72	-27.53	-31.75	-32.43
Maccaffertium mediopunctatum	-26.96	-27.14	-29.49	-28.56
Optioservus sp.	-28.16	-28.72	n.a.	n.a.
Psephenus herricki	n.a.	-24.38	-28.72	-31.67
Pteronarcys dorsata	-32.32	-30.13	-32.67	-34.12
Pycnopsyche sp.	-27.38	-28.24	-28.64	-30.01
Serratella sp.	n.a.	-32.50	n.a.	n.a.
Simulium sp.	-27.11	-25.71	-35.38	-36.31
Stenacron interpunctatum	n.a.	-27.12	n.a.	n.a.
Stenelmis crenata group	-28.32	-28.79	-28.75	-30.53
$\delta^{15}N$				
Cheumatopsyche sp.	8.75	8.56	8.69	n.a.
Corbicula fluminea	10.23	9.66	8.65	9.39
Hydropsyche simulans	9.33	9.12	9.04	10.31
Isonychia sp.	9.39	8.77	8.42	7.56
Lepidostoma sp.	9.18	7.22	6.88	n.a.
Leptoxis praerosa	9.56	9.55	8.71	8.32
Maccaffertium mediopunctatum	8.23	7.49	6.73	6.30
Optioservus sp.	8.90	8.66	n.a.	n.a.
Pteronarcys dorsata	8.40	7.83	7.78	8.11
Psephenus herricki	n.a.	6.31	8.10	6.22
Pycnopsyche sp.	9.69	8.98	7.73	7.22
Serratella sp.	n.a.	7.91	n.a.	n.a.
Simulium sp.	8.98	8.83	7.59	8.25
Stenacron interpunctatum	n.a.	9.08	n.a.	n.a.
Stenelmis crenata group	8.56	8.30	7.58	6.95

Table 3: Mean and range of $\delta^{13}C$ values of macroinvertebrate communities from the upper Green River during summer 2012. Reaches are listed in order of upstream to downstream. Niche breadth was measured by the range of $\delta^{13}C$ values (Layman *et al.* 2007).

Reach	**mean $\delta^{13}C$**	**mean $\delta^{15}N$**	**$\delta^{13}C$ range**	**Niche breadth**
A	-27.92	9.10	-32.32 – -24.04	8.28
B	-27.95	8.42	-32.50 – -24.38	8.12
C	-30.65	7.99	-35.38 – -28.64	6.74
D	-31.44	7.86	-36.31 – -28.56	7.75

Table 4: Carbon and nitrogen isotope data from potential food resources collected from the upper Green River during autumn 2013. Reaches are listed in order of upstream to downstream. n.a. = resources not collected from a study reach.

Isotopic ratio	**Reach**			
$\delta^{13}C$	A	B	C	D
Cladophora	-34.75 ± 0.02	-43.34 ± 0.01	-44.00 ±0.01	-35.95 ± 0.06
P. ceratophyllum	-36.64 ± 0.01	-36.39 ± 0.02	-39.11 ±0.03	-38.31 ± 0.05
composite leaves	-29.92 ± 0.28	-29.92 ± 0.28	-30.22 ±0.18	-30.22 ± 0.18
composite TOM	-28.70 ± 0.06	-26.80 ± 0.30	-29.10 ±0.18	-25.25 ± 0.04
composite biofilm - wood	-27.64 ± 0.21	-27.90 ± 0.05	-27.54 ±0.09	-27.29 ± 0.04
composite biofilm - rock	-20.52 ± 0.21	-11.93 ± 0.14	-29.94 ±0.08	-25.14 ± 0.01
BOM - fine	-27.07 ± 0.02	-26.18 ± 0.01	-26.33 ±0.01	-26.33 ± 0.01
BOM - coarse	-28.13 ± 0.06	-28.27 ± 0.09	-27.86 ±0.02	-27.86 ± 0.02
$\delta^{15}N$				
Cladophora	8.24 ± 0.02	6.28 ± 0.02	5.53 ± 0.01	6.88 ± 0.01
P. ceratophyllum	7.96 ± 0.03	8.73 ± 0.01	8.40 ± 0.04	10.28 ± 0.02
composite leaves	0.83 ± 0.28	0.83 ± 0.28	2.31 ± 0.30	2.31 ± 0.30
composite TOM	5.43 ± 0.47	5.93 ± 0.15	4.76 ± 0.28	6.31± 0.05
composite biofilm - wood	6.92 ± 0.31	6.76 ± 0.08	5.99 ± 0.04	7.05 ± 0.09
composite biofilm - rock	7.24 ± 0.17	7.52 ± 0.25	5.99 ± 0.05	7.97 ± 0.03
BOM - fine	6.03 ± 0.08	6.80 ± 0.08	5.97 ± 0.11	5.97 ± 0.11
BOM - coarse	4.61 ± 0.03	5.21 ± 0.15	4.67 ± 0.15	4.67 ± 0.15

TOM = transported organic matter, BOM = benthic organic matter

Table 5: Carbon and nitrogen isotope data from common primary consumer taxa collected from the upper Green River during autumn 2013. Reaches are listed in order of upstream to downstream. n.a. = resources not collected from a study reach.

Isotopic ratio	**Reach**			
$\delta^{13}C$	A	B	C	D
Cheumatopsyche sp.	n.a.	-31.07 ± 0.40	n.a.	n.a.
Corbicula fluminea	-31.32 ± 0.18	-30.74 ± 0.06	-29.62 ± 0.02	-30.73 ± 0.12
Hydropsyche simulans	-29.02 ± 0.02	-29.54 ± 0.02	-32.24 ± 0.03	-28.49 ± 0.01
Isonychia sp.	-29.92	-29.79	n.a.	-30.14 ± 0.02
Lepidostoma sp.	n.a.	n.a.	-33.48 ± 0.01	-32.83 ± 0.01
Leptoxis praerosa	-26.68 ± 0.05	-27.89 ± 0.07	-30.16 ± 0.01	-28.54 ± 0.04
Maccaffertium mediopunctatum	-28.99 ± 0.06	-29.46	-29.99 ± 0.03	n.a.
Optioservus sp.	-28.86 ± 0.05	-28.66 ± 0.04	-29.40 ± 0.15	-28.46 ± 0.02
Pleuroceridae - smooth shell	n.a.	n.a.	-27.90 ± 0.04	-27.60 ± 0.04
Pleuroceridae - wrinkled shell	-24.85 ± 0.06	n.a.	-26.89 ± 0.03	-28.04 ± 0.11
Psephenus herricki	-25.95 ± 0.03	-21.81 ± 0.04	-30.30 ± 0.06	-31.37 ± 0.07
Pteronarcys dorsata	n.a.	-32.94 ± 0.05	-33.59 ± 0.07	-33.31 ± 0.01
Pycnopsyche sp.	n.a.	-29.06 ± 0.03	-32.44 ± 0.04	-30.19 ± 0.01
Stenacron interpunctatum	n.a.	-26.41 ± 0.01	n.a.	n.a.
Stenelmis crenata group	-29.09	n.a.	-29.99	-30.80 ± 0.03
$\delta^{15}N$				
Cheumatopsyche sp.	n.a.	8.91 ± 0.17	n.a.	n.a.
Corbicula fluminea	10.65 ± 0.16	9.58 ± 0.03	8.88 ± 0.07	9.38 ± 0.06
Hydropsyche simulans	10.16 ± 0.02	10.81 ± 0.02	9.26 ± 0.08	10.76 ± 0.03
Isonychia sp.	9.41	8.05	n.a.	8.61 ± 0.02
Lepidostoma sp.	n.a.	n.a.	8.67 ± 0.05	5.91 ± 0.06
Leptoxis praerosa	10.23 ± 0.07	10.38 ± 0.09	9.74 ± 0.04	9.72 ± 0.02
Maccaffertium mediopunctatum	9.07 ± 0.03	9.53	7.95 ± 0.04	n.a.
Optioservus sp.	8.75 ± 0.07	9.00 ± 0.12	8.16 ± 0.11	7.38 ± 0.11
Pleuroceridae - smooth shell	n.a.	n.a.	9.32 ± 0.05	9.97 ± 0.04
Pleuroceridae - wrinkled shell	10.33 ± 0.05	n.a.	9.86 ± 0.04	9.70 ± 0.09
Psephenus herricki	7.96 ± 0.04	7.96 ± 0.09	6.32 ± 0.02	7.84 ± 0.05
Pteronarcys dorsata	n.a.	9.95 ± 0.09	8.27 ± 0.04	8.02 ± 0.04
Pycnopsyche sp.	n.a.	9.16 ± 0.02	8.82 ± 0.08	8.16 ± 0.02
Stenacron interpunctatum	n.a.	9.86 ± 0.02	n.a.	n.a.
Stenelmis crenata group	8.89	n.a.	6.95	6.65 ± 0.10

Table 6: Mean and range of $\delta^{13}C$ values of macroinvertebrate communities from the upper Green River during autumn 2013. Reaches are listed in order of upstream to downstream. Niche breadth was measured by the range of $\delta^{13}C$ values (Layman *et al.* 2007).

Reach	**mean $\delta^{13}C$**	**mean $\delta^{15}N$**	**$\delta^{13}C$ range**	**Niche breadth**
A	-28.30	9.50	-31.32 – -24.85	6.47
B	-28.85	9.38	-32.94 – -21.81	11.13
C	-30.50	8.52	-33.59 – -26.89	6.70
D	-30.25	8.62	-33.31 – -27.60	5.72

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